

Animal Models of Immunological Processes

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1982



ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

LONDON NEW YORK

PARIS SAN DIEGO SAN FRANCISCO

SYDNEY TOKYO TORONTO

SÃO PAULO

2 Lymphocyte Locomotion, Lymphatic Tissues and Lymphocyte Circulation in the Rat*

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1. Introduction

Gowans and his collaborators (1957, 1959) provided the first convincing evidence that lymphocytes constantly travel from the blood stream into lymphatic tissues and return to the circulation via major efferent lymphatics. Success of the rat model depended upon the availability of highly inbred strains (Heslop and Hardy, 1971), small animal surgical techniques for thoracic duct cannulation (Bollman *et al.*, 1948), and radionucleotide labeling methods for tracing tagged lymphocytes *in vivo* (Gowans, 1959). The phenomenon of recirculation is now regarded as representative of normal lymphocyte behavior in most mammals, including man.

Lymphocytes can be divided into two classes with distinctly different functions. In birds, cellular immunity is dependent upon thymus-derived lymphocytes, while humoral antibody responses are mediated by lymphocytes originating in the bursa of Fabricius (Glick, 1956). The analogue for the bursa has not been identified in other species, but it is generally believed that the liver and bone marrow serve this function in immature and adult mammals, respectively (Cooper *et al.*, 1973). This division of labor is not so rigid as originally believed. Miller (1962a, b) found that neonatal thymectomy in rodents impaired both cellular

*The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

and humoral responses, and infusions of thoracic duct lymphocytes or thymic grafts (Osoba and Miller, 1964) corrected these defects. The T cell contribution to humoral immunity was revealed by subsequent observations that both thymus and bone marrow cells were needed to restore humoral antibody responses in irradiated mice (Mitchell and Miller, 1968; Claman and Chaperon, 1969). The demonstration by karyotype analysis that Jerne plaque-forming cells from such mice were of bone marrow and not thymic origin (Nossal *et al.*, 1968b) promoted the concept that T cells played important "helper cell" functions in humoral immunity. It is currently believed that T cell help is required for most humoral antibody responses (Mitchison, 1971; Jacobson and Herzenberg, 1972; Herzenberg *et al.*, 1973; Kishimoto and Ishizaka, 1973), but some repeating-polymer antigens, such as pneumococcal polysaccharide, may directly stimulate B cells. T cell help is also believed to be important in the triggering and differentiation of cytotoxic T cells, nonspecific suppressor cells, and antigen-specific suppressor cells (Shevach, 1981).

Since soluble factors released by immunoregulatory cells can mediate amplification or suppression of immunity, it is not clear whether helper or suppressor T cells need to directly contact the respective effector cell in order to produce their effects. In the case of B cell responses, antigens are bound to the surfaces of specifically reactive cells via immunoglobulin molecules which are inserted in the membrane; such antigen-binding activity has been difficult to demonstrate for T cells. Apparently the T cell antigen receptor is not an immunoglobulin and is either secreted into the surrounding environment (Binz and Wigzell, 1975a, b) or is weakly held by the lymphocyte membrane. Since T cells depend heavily on Ia-antigen-bearing macrophages for antigen reactivity, it is possible that macrophages serve an antigen-binding role for T cells by nonspecifically absorbing onto their surfaces the released T cell receptor-antigen complexes as predicted by Feldman (1973) in his IgT model. Nonphagocytic dendritic (Steinman *et al.*, 1975; Stenman and Vaheri, 1978) and Langerhans cells (Katz, 1977) apparently serve this antigen-binding presentation role as well.

Indications of functional heterogeneity among the classes of lymphocytes and evidence that various kinds of cellular interactions were highly restricted contributed to rather than weakened the status of lymphocyte recirculation as an immunological phenomenon. Considering the tremendous diversity of antigens in the environment and the "one-cell one antigenic determinant" tenet of the clonal selection theory of immunity (Burnet, 1959), it is difficult to see how the

appropriate cells would be in the same place at the same time as antigen without a dynamic process-like lymphocyte recirculation. This constant flux and sorting of immunocompetent lymphocytes guarantees that a significant portion of the total uncommitted lymphocyte population will traffic past antigen/macrophage depots in lymphatic tissues. Studies by Gowans (1959) and Ford (1969) indicate that the tempo of recirculation is sufficient to replace the blood pool of lymphocytes ten times daily and up to 20% of the lymphocytes in each lymph node in the same period. It was initially believed that only T cells recirculated but recent studies by Howard *et al.* (1972), Ford and Simmonds (1972), and Sprent (1977) have indicated that both T and B lymphocytes recirculate from blood to lymph; however, B cells incorporated ³H-uridine less intensely than T cells; B cells transit lymphatic tissues more slowly than T cells. Radioautographic evidence of differential uptake of tritiated uridine by T and B cells has been useful in enumerating and tracing the migratory populations of these respective cells in lymphatic tissues (Howard *et al.*, 1972; Anderson and Reynolds, 1979). Human T cells also label more heavily than B cells, thereby contributing to the model characteristics of the rodent immune system for similar phenomena in humans.

Given these vital functions of recirculating lymphocytes in immunity, what mechanisms control the homing, emigration, and recirculation of immunocompetent cells *in vivo*? This chapter will attempt to answer this question by discussing: (a) factors which are intrinsic to the migrating cells, such as spontaneous locomotory behavior, cytoskeleton-mediated intrinsic motility, and ability to exhibit chemotaxis; (b) factors which control *in vivo* distribution, such as regional blood flow and cell-surface recognition phenomena; (c) effects of the development and differentiation of lymphocytes and lymphatic tissues on lymphocyte migration and distribution; and (d) mechanisms controlling the migration patterns and tissue distribution of lymphocytes in normal and antigenically stimulated adult lymphatic tissues.

II. Intrinsic migratory behavior of "recirculating" lymphocytes

It has been known for nearly 50 years that lymphocytes are motile cells which exhibit cycles of spontaneous movement interrupted by resting stages of variable length (Lewis, 1931). However, migration is executed by lymphocytes only with great difficulty because of a relative deficiency of the cytoplasmic machinery for locomotion (McCutcheon, 1946). The high nuclear:cytoplasmic ratio is probably responsible for

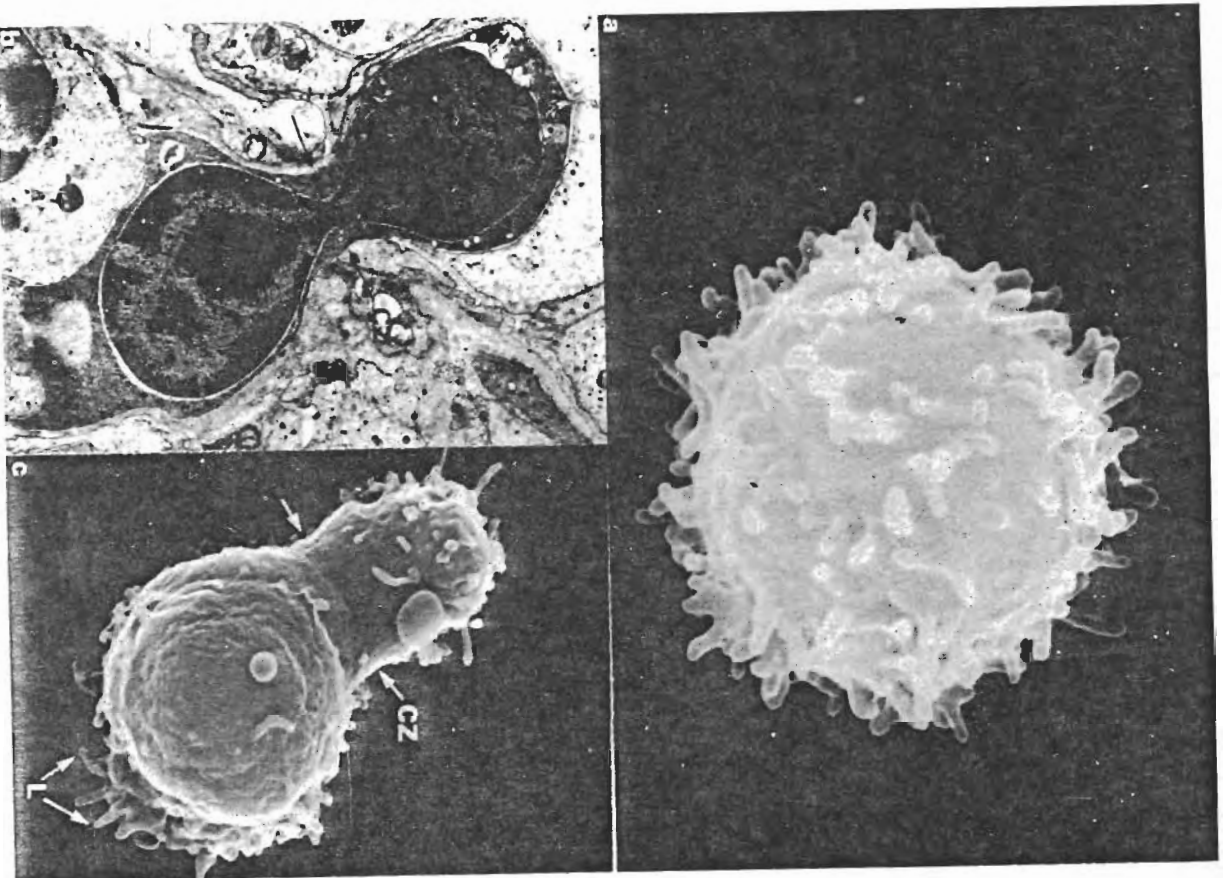


FIG. 1 (a) Normal T and B lymphocytes isolated from thoracic duct lymph bear microvilli on their surfaces. (b) and (c) Lymphocytes which are locomoting lose their microvilli over the zone of constriction (CZ) as lamellipodia (L) form anteriorly and microvilli cluster at the end of the uropod (arrow).

the characteristic morphology of locomoting lymphocytes observed by Lewis (1931), Harris (1954), and others. Resting lymphocytes isolated from thoracic duct lymph or peripheral blood are spherical cells with uniformly distributed microvilli on their surfaces (Fig. 1). Between 15.6 and 30% of these cells spontaneously move during 10 min of observation of coverslip preparations warmed to 37°C. As migration begins, the lymphocyte probes its surroundings by extending and retracting villous projections and lamellipodia. Adhesion of a lamellipodium to the substratum causes the cell to elongate into the characteristic "hand-mirror" shape with anterior nucleus and posterior uropod. The lymphocyte continuously extends lamellipodia anteriorly and appears to drag its uropod like a "ball and chain" as it contracts and relaxes through cycles of translocation. Waves of cytoskeletal contraction constrict the nucleus and squeeze it into the anterior end of the cell as each contraction propagates from front to rear. The constriction zone, which contains a circumferentially thickened sub-plasmalemmal microfilament network, remains stationary with respect to the substratum as the rest of the cell moves forward (Fig. 1). The cell surface overlying the constriction zone is devoid of microvilli when examined by scanning electron microscopy (Anderson *et al.*, 1979). Both T and B lymphocytes exhibit spontaneous motility, although rates of locomotion vary. Small T lymphocytes from thoracic duct lymph travel at a mean velocity of $11.9 \mu\text{m min}^{-1}$ while B cells move more slowly ($5.3 \mu\text{m min}^{-1}$), if at all. B cells appear to require some stimulation before they move. Exposure of B cells to anti-immunoglobulin induces capping, which is followed by translocation (Schreiner and Unanue, 1975). B cells also begin to move after they have been fondled by a migrating T cell. Blast cells of either class move faster than small lymphocytes, their average speed being $24 \mu\text{m min}^{-1}$. While the migration characteristics of lymphocytes do not appear to be as great as those of neutrophils and monocytes, which move at rates between 30 and $70 \mu\text{m min}^{-1}$, what movement lymphocytes can manage is obviously sufficient to transport these immunocompetent cells across the vascular barrier into the reticular meshworks and lymphatic channels of lymph nodes, Peyer's patches and spleen (Fig. 2). Global redistribution of ligands bound to surface receptors ("capping") is another manifestation of lymphocyte motility (Schreiner and Unanue, 1976). Most capping phenomena appear to involve participation of the cytoskeleton, protein synthesis and cellular respiration, since drugs which affect these activities inhibit capping. However, in rare instances, capping may also result from passive diffusion and aggregation of cross-linked receptors within the fluid

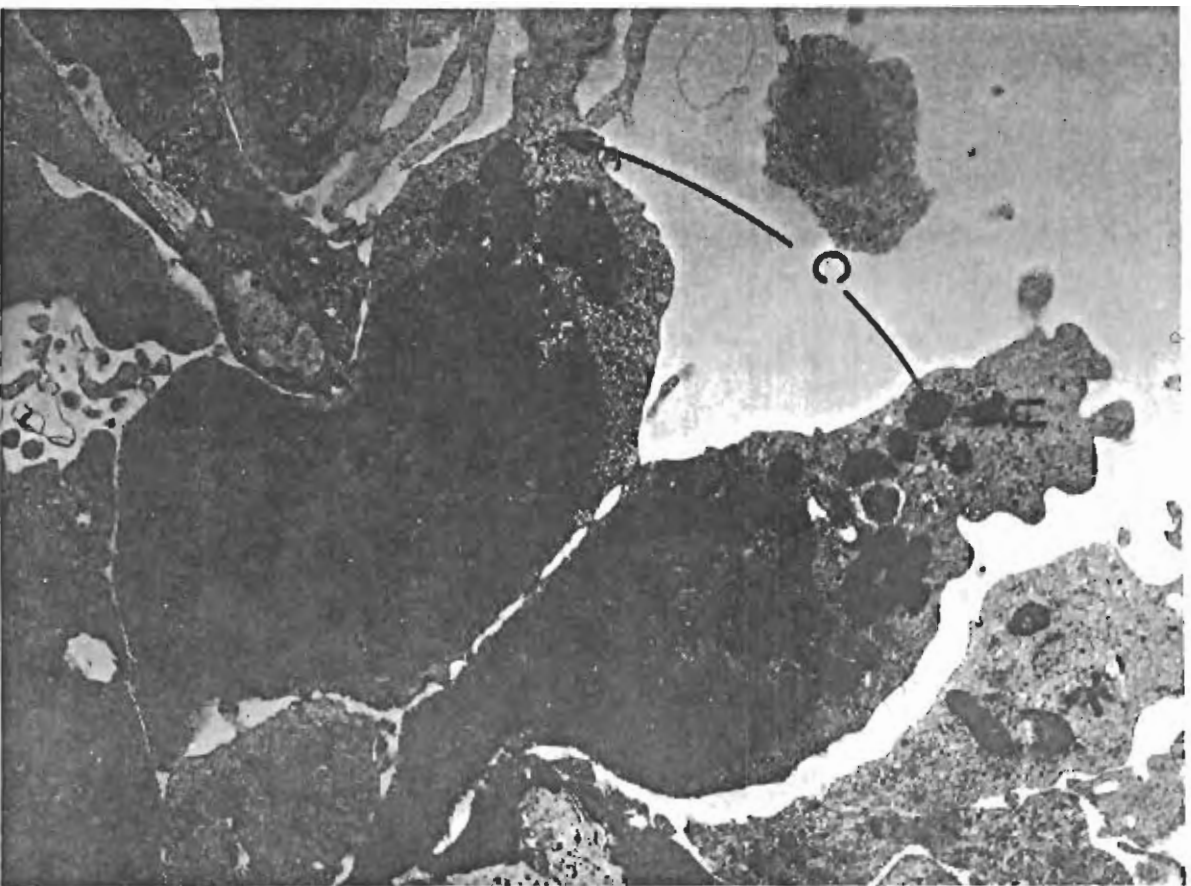


FIG. 2 The cytoplasm of locomoting lymphocytes is polarized. The organelle-rich uropod (U) containing the centriole (C) is the terminus of a symmetrical contraction wave which propagates anteroposteriorly around the nucleus as the cell moves forward.

membrane. The capping of membrane-associated immunoglobulin molecules by multivalent anti-immunoglobulin antibodies apparently is an inductive signal, which precipitates B cell locomotion, because it frequently is followed by translational movement. Regardless of whether the B cell caps or locomotes first, the anti-Ig cap is usually found at the end of the uropod of migrating cells. This is also true of the red cells stuck to locomoting human T cells, which had been incubated with sheep erythrocytes (Fig. 3). These phenomena suggest that oriented movement of surface receptors occurs from front to rear during locomotion. This is not necessarily true during all cases of capping, since certain ligands form caps which are not localized over

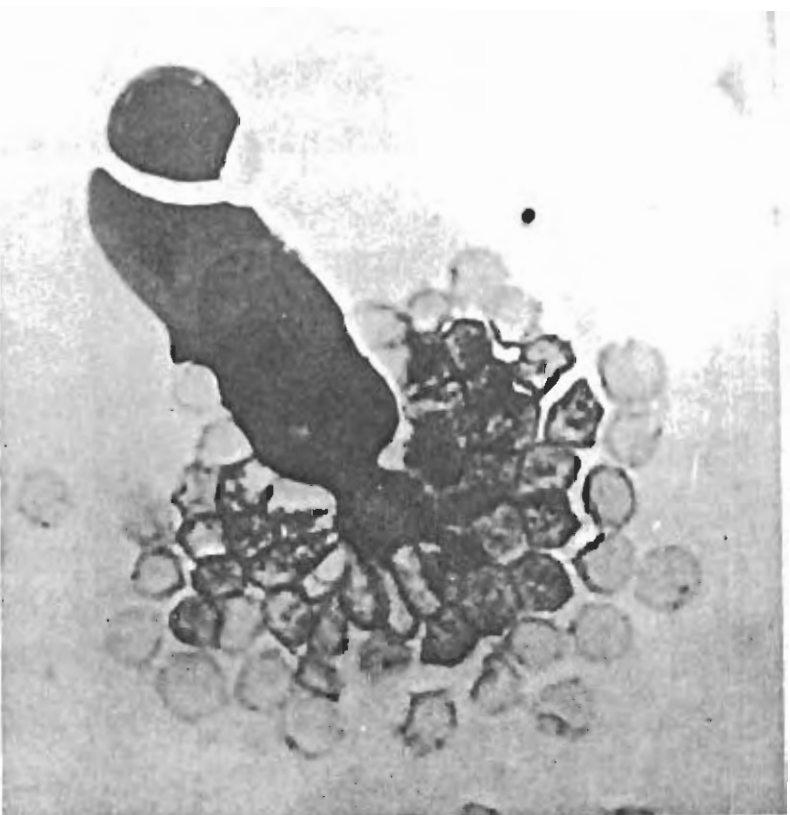
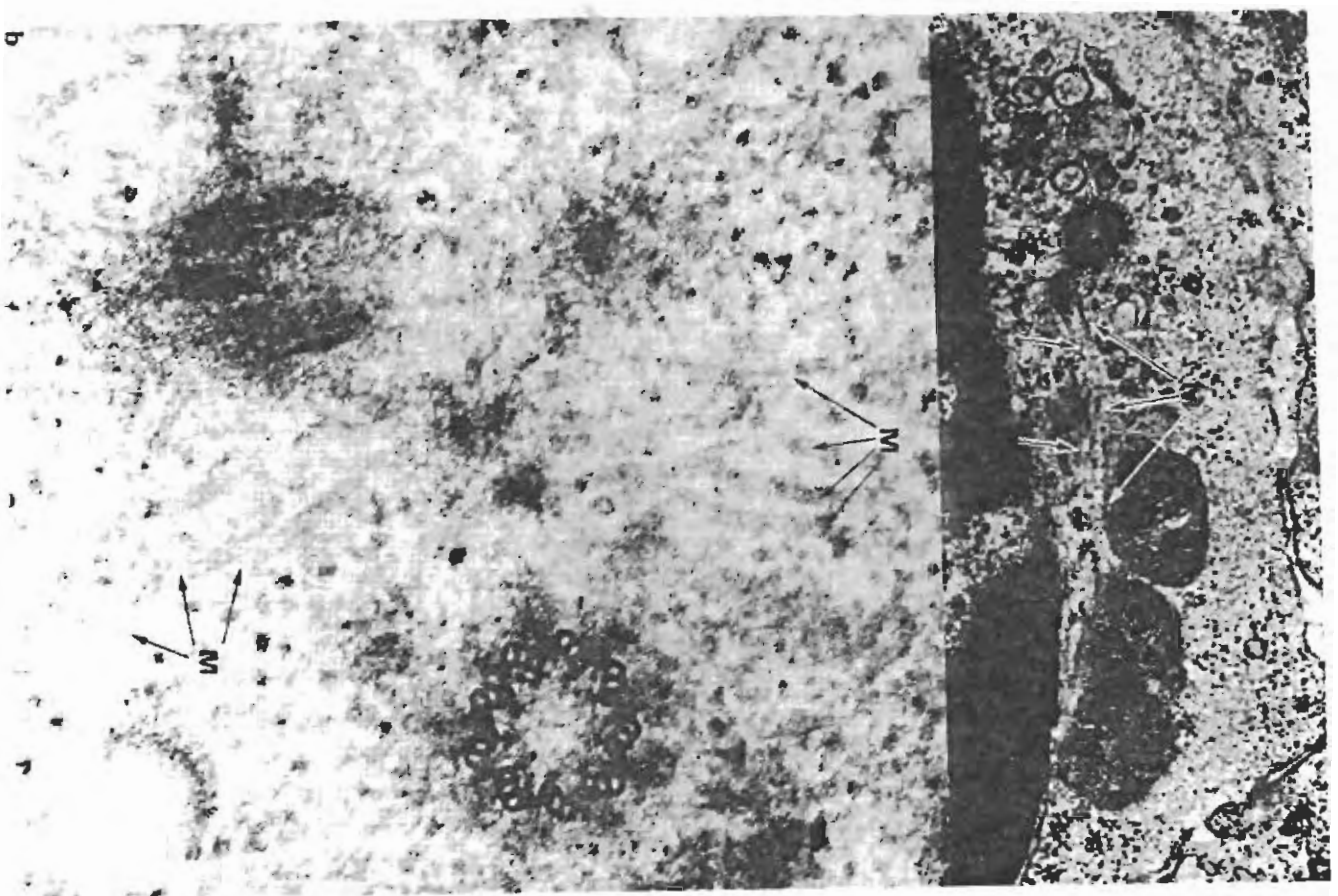


FIG. 3 Human lymphoblasts (generated during 7-day culture in 5% fetal calf serum) avidly bind and cap sheep erythrocytes (Michalski *et al.*, 1980).



the centriole or Golgi zone of the cell. Since the centriole is always found in the uropod end of locomoting and capping lymphocytes, it is likely that this structure is responsible for the cytoplasmic and cytoskeletal polarization necessary for these motile functions. A primary cilium generated by the centriole appears to serve this orienting function in other types of eukaryotic cells. The organization of the lymphocyte cytoskeleton is still uncertain, but an umbrella-like network of microtubules (Fig. 4) may be seen radiating from the centriole around the nucleus toward the lamellipodia of lymphocytes which have become polarized prior to locomoting (Anderson *et al.*, 1978a, b). A network of 5-nm filaments forms a continuous mat beneath the plasmalemma and is arranged within the core of microvilli as longitudinally oriented fibers. Ten-nanometer filaments appear to extend anteriorly beside the microtubules and can be seen forming loops in the lamellipodia of motile cells. The complex interrelationships of these fibers during various biological activities remain to be determined.

The directed migration of leukocytes along a chemical gradient is generally accepted as playing an important *in vivo* role in regulating cellular traffic and promoting the accumulation of inflammatory cells at sites of tissue injury. Historically, lymphocytes were not regarded as being capable of chemotaxis, because of their erratic behavior in crude coverslip chambers (McCutcheon, 1946; Harris, 1954). Recent evidence indicates that mitogen-stimulated T lymphoblasts and some lines of transformed lymphocytes display chemotaxis toward casein hydrolysates, denatured albumin, endotoxin-activated serum, submitogenic doses of plant and microbial lectins (Russell *et al.*, 1975; O'Neill and Parrott, 1977; Wilkinson *et al.*, 1977). In addition, supernatants isolated from mixed lymphocyte cultures, mitogen-stimulated lymphocytes and activated macrophages have been said to evoke chemotaxis by spleen and lymph node cells (Ward *et al.*, 1971, 1977). Many of these studies were conducted in systems which cannot distinguish between chemotaxis (directed migration of cells which are oriented by a gradient) and chemokinesis (increased random migration which is greatest on the side facing the gradient), and each of these factors are equally chemoattractive to other leukocytes. Therefore, it is possible that true chemotaxis cannot be demonstrated using single-

FIG. 4 (a) and (b) Microtubules (M) and 10-nm filaments (arrows) radiate from the centriole, around the nucleus, in polarized lymphocytes. The thin section shown in (a) is oriented longitudinally from left to right, while (b) is a coronal section through the centriolar region.

parameter assays. Preliminary observations using a "three-chamber" agarose system (Anderson and Warren, 1979) suggest that thoracic duct lymphocytes (TDL) are inefficient in their ability to orient in a gradient of endotoxin-activated serum presented to them by diffusion

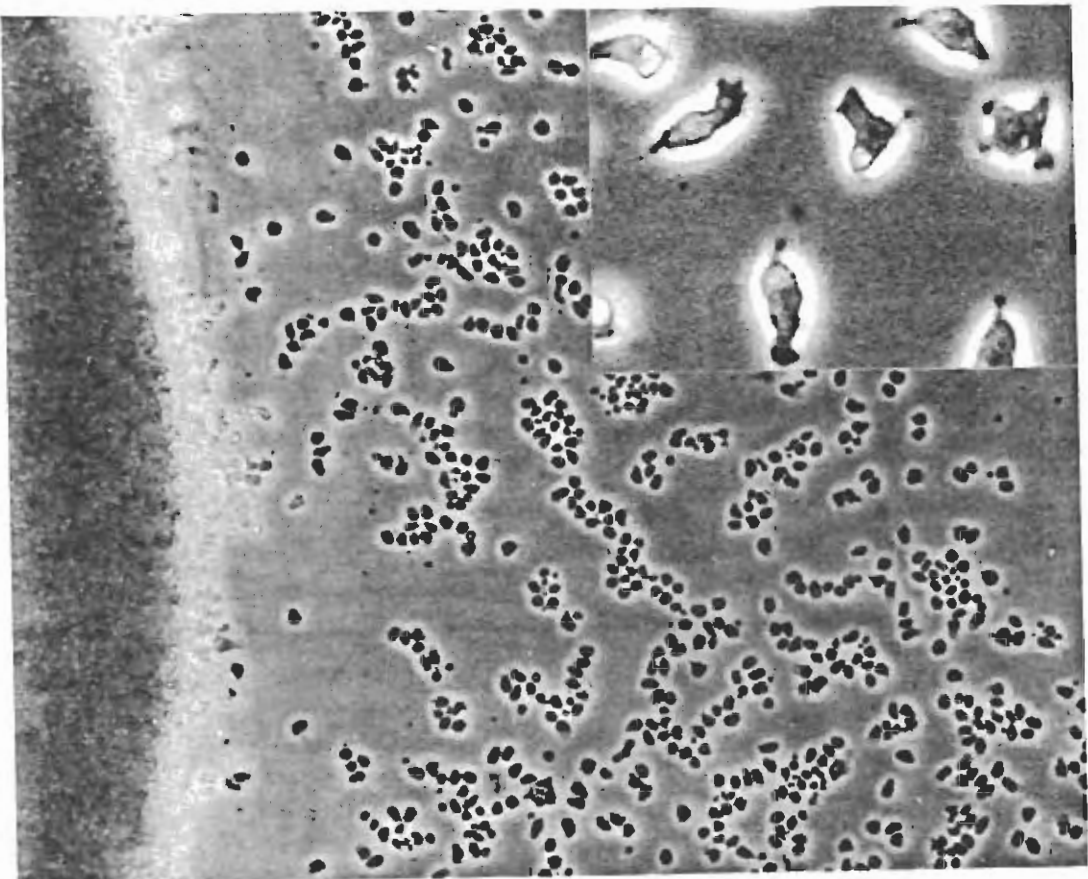


FIG. 5 Thoracic duct lymphocytes crawl beneath agarose for distances ranging from 80 to 60 μm during 18-24 hours' incubation at 37°C in 5% CO_2 . Inset: high-power view of locomoting live lymphocytes.

through agarose. However, TDL populations show significant net orientation and directional migration toward such a gradient when compared to random migration controls (Fig. 5). Closer examination of the cellular interactions among these slightly heterogeneous populations indicates that T lymphocytes appear to be about as attracted to nearby B lymphocytes and small macrophages as they are to the artificial gradient (Anderson and Warren, 1980). This local interference in gradient-sensing by cellular chemotaxins is probably responsible for the highly erratic migration patterns of lymphocytes moving into chemotactic gradients (Fig. 6). Since directed lympho-

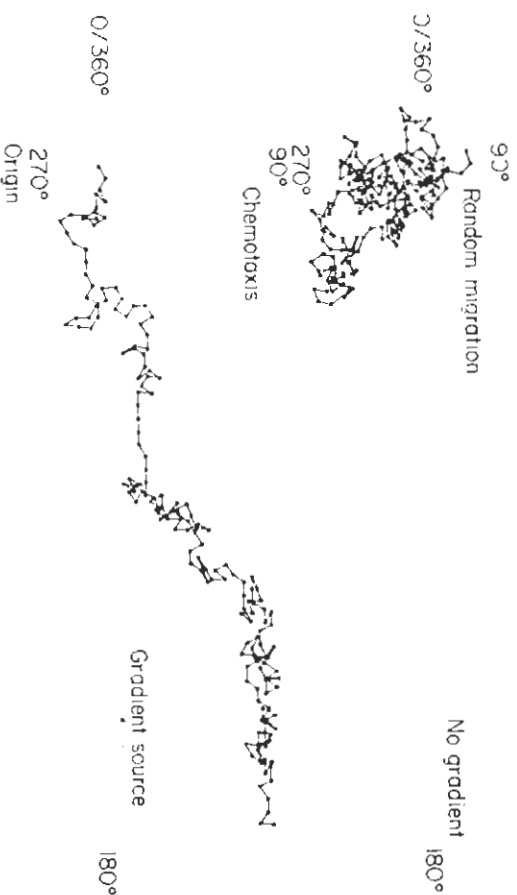


FIG. 6 Randomly migrating lymphocytes and lymphocytes which show directed movement toward a "chemotactic" gradient exhibit erratic migration paths. Lymphocytes are capable of correcting enough of the erroneous orientations to make progress toward a gradient of endotoxin-activated serum. The deviations in the course appear to be caused by locally released "factors" generated by the B cells and/or macrophages present in TDL. (Anderson and Warren, 1980.)

cytes frequently reverse or change orientations by 90° increments, while making net movement toward the gradient source, the chemotropism index for chemotaxing lymphocytes is 0.45-0.50. This is what one would expect of a randomly moving neutrophil. However, lymphocytes moving in the absence of a gradient, reverse direction so often that they fail to accumulate any net forward movement and

produce chemotropism indices between 0.09 and 0.12. Further studies are needed to determine whether chemotaxis plays any role in regulating the characteristic traffic patterns displayed by lymphocytes *in vivo*. Specialized microvascular structures at lymphatic tissue/blood interfaces may have developed during evolution to compensate for the relative inability of lymphocytes to display efficient "goal-directed" forward movement. Diffusion of small quantities of factors produced by macrophages, antigens, immunoblasts and concentrations of other lymphocytes into vascular lumens may be all that is needed to provoke the emigration of a circulating lymphocyte. Once inside, other factors and interactions with other cells apparently determine its ultimate tissue distribution (Curtis and de Sousa, 1973).

III. Lymphocyte homing to HEV

It has been widely observed that the proportion of lymphocytes to other blood elements is incredibly high in the lumens of high endothelial venules of lymph nodes (Zimmerman, 1923; Schulze, 1925). Early anatomical studies attributed this accumulation to movement of lymphocytes generated in the node into the blood stream at these sites. However, numerous radiolabel tracer studies by Gowans and colleagues clearly indicated that the traffic was from blood to lymph. Since uridine strongly labels T cells but only weakly B cells, it is possible that some cells (perhaps a population which goes unlabeled) could migrate in opposite directions or return to the blood directly after a rapid sojourn in the connective tissue sheath of the HEV (Sainte-Marie and Sin, 1970). Other mechanisms were proposed to explain the accumulation of lymphocytes in HEV lumens, however. Schulze (1925) postulated that stagnation of blood flow produced by the sudden transition in vascular diameter as narrow capillaries emptied into wide HEV lumens could result in concentration of circulating lymphocytes within these venule lumens, while others concluded that lymphocytes in the circulation bumped into HEV endothelial cells which protruded into the lumen.

A. THE HOMING RECEPTOR

There seems to be little reason for doubting that slowing of venous flow through HEV might facilitate lymphocyte endothelial cell interactions, since lymph nodes contain a microvasculature which is capable of

rather extreme changes in hemodynamics (Anderson *et al.*, 1975; Hay and Hobbs, 1977). Attempts to dislodge adherent lymphocytes by flushing with arterial perfusates revealed that 75% of the luminal lymphocytes remained attached to endothelial surfaces (Van Ewijk *et al.*, 1975; Anderson and Anderson, 1976). *In vivo* studies of leukocyte sticking in other vascular beds (Atherton and Born, 1972) indicated that luminal adhesion depends upon the net forces of cellular adhesion and hemodynamic shearing. Under these conditions accelerated blood flow through HEV might prevent homing, while sluggish flow might facilitate surface interactions. Since other blood leukocytes rarely are found adhering to HEV surfaces or emigrating across their walls, several investigators (Gowans and Knight, 1964; Marchesi and Gowans, 1964; Goldschneider and McGregor, 1968; Sordat *et al.*, 1971; Schoel, 1972) suggested that selective movement of lymphocytes across these venules was dependent on cell-surface recognition mechanisms. Although HEV possess characteristic structural and metabolic features (Anderson *et al.*, 1976), none of these has been directly shown to regulate cellular traffic. However, scanning electron microscopy (SEM) studies demonstrated that the luminal surface of HEV was studded with shallow pits which morphologically resembled so-called "coated pits" on transmission electron microscopy (TEM) sections. While these structures might be seen on other vascular surfaces, their size and distribution on HEV were more prominent. Lymphocytes attached to these surface foci via microvilli which deeply indented the endothelial surface (Fig. 7, 8). Since EM preparations using mordants which preserved surface oligosaccharides revealed membrane specializations and electron-dense cross-bridging between lymphocyte and endothelial cell membranes, it is proposed that this represented some form of homing "receptor" complex. The presence of complementary surface receptors for homing on lymphocytes was first suggested by Gesner and Ginsburg (1964) and Woodruff and Gesner (1968), when they found that removal of membrane glycoproteins by *in vitro* trypsin digestion prevented lymphocytes from homing to HEV. Subsequent studies by Woodruff (1974) indicated that 24 h later these cells had resynthesized their receptors and thus were able to home normally to HEV. Reciprocal studies (Anderson and Anderson, 1976) where the lymph node microvasculature was perfused with 0.1% trypsin indicated that all adherent lymphocytes could be dislodged without affecting HEV surface coat or structural integrity. These studies did not take into consideration the cytoplasmic effects of external trypsin treatment (i.e. release of microfilament attachments to membrane by digestion of alpha actinin and "activation" of the

cyclic AMP system by trypsin treatment); nevertheless, it seemed likely that both cellular recognition and attachment were dependent upon interactions between membrane glycoproteins on lymphocytes and endothelial cells. Perfusion studies also indicated that an EDTA-chelatable divalent cation was important in lymphocyte adhesion, possibly by forming cationic bridges between negatively charged carboxyl or sulfate groups on apposing membranes (Bangham, 1964). Since strongly anionic linear polymers, such as heparin or dextran

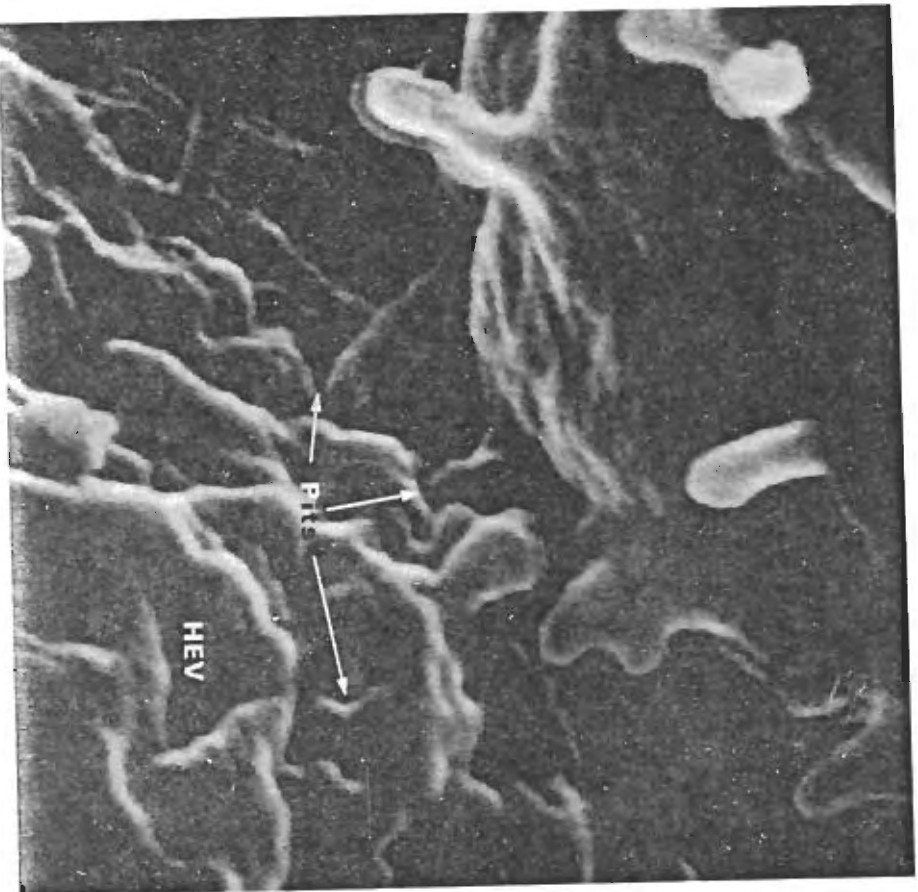


FIG. 7 In perfusion-fixed scanning electron microscopic preparations of lymph node high endothelial venules (HEV), lymphocytes remain adherent to endothelial surfaces via microvilli which interact with "pits" on the endothelial cells.

sulfate, appear to block lymphocyte homing without damaging these cells or resulting in sequestration in RE organs, it is possible that electrostatic linkages (Ford *et al.*, 1978) between "selective" oligosaccharide groups may be one mechanism for lymphocyte homing, but the issue remains open for the time being.



FIG. 8 Transmission electron microscopy studies indicate that this villus-pit interaction provides sufficient interdigitation to prevent the hydrodynamic shear forces of perfusates from dislodging the cells.

B. ROLE OF ACTIVE MOTILITY IN LYMPHOCYTE HOMING AND EMIGRATION *IN VIVO*

Recirculating lymphocytes emigrate from the blood into lymph nodes by selectively adhering to the endothelial surfaces of HEV and crossing the walls by active translocation. This process depends on the existence of membrane receptors for attachment to endothelium and on the capacity of the lymphocytes to propel themselves across HEV. Microfilaments and microtubules may be involved in both of these aspects of

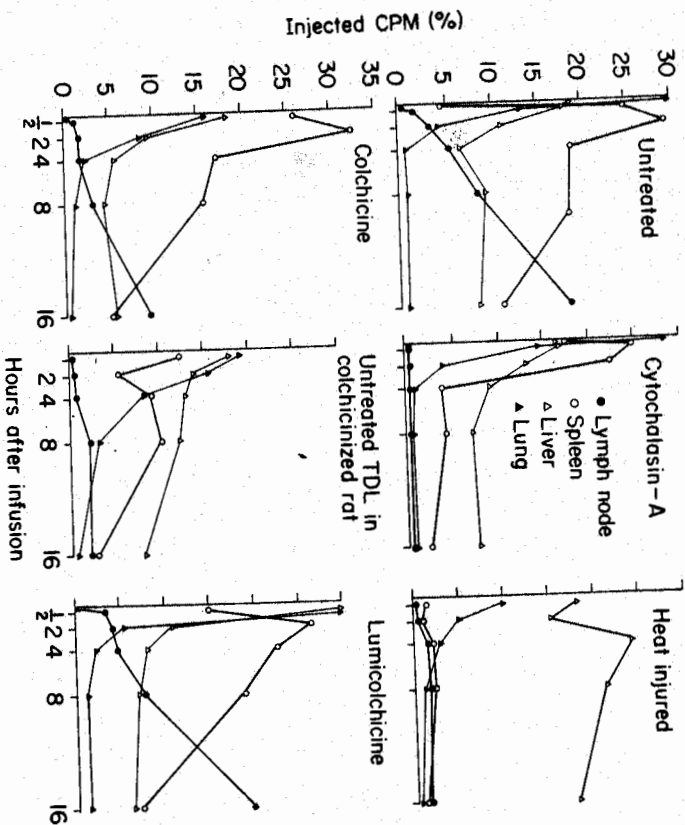


FIG. 9 There is a rapid accumulation of label in the lung, liver and spleen immediately after transfusion of 4×10^8 normal ^3H -uridine-labeled lymphocytes. The magnitude of initial accumulation of label is proportional to the percentage of the cardiac output flowing through each organ. The spleen normally skins off large numbers of lymphocytes within its blood-filtering reticulum and the cell accumulation naturally peaks early. The radioactivity in the spleen after 2 hours represents semi-selective migration of lymphocytes into the perarteriolar lymphatic sheath (PALS). Some lymphocytes are returned without ever coming into contact with the PALS. The progressive accumulation of label in lymph nodes is generally regarded as an indication of "selective emigration" from the blood. TDL treated with $4.0 \mu\text{g ml}^{-1}$ CA failed to accumulate in lymph nodes in appreciable numbers, and splenic uptake was also reduced. Liver uptake of CA-treated lymphocytes was not different from normal, which attested to the viability of CA-treated TDL. Heat-killed lymphocytes were sequestered in the liver by Kupfer cells at the expense of all other tissues. Lymphocytes treated with 10^{-4} M colchicine showed depressed accumulation in lymph nodes, which was significantly reduced until 8 hours after infusion. When untreated TDL were infused into rats given intraperitoneal colchicine at $1.0 \mu\text{g g}^{-1}$ body weight, both lymph node and splenic uptake was depressed. In contrast, 10^{-4} M lumicolchicine-treated TDL migrated normally when infused into untreated rats.

emigration, since the receptors for homing may depend upon trans-membrane linkages to cytoskeletal structures for clustering or distribution of these receptors on microvilli or in patches of specialized membrane, and receptor complexes may be transported through the membrane along the longitudinal axis of the cell during translocation. Doses of cytochalasin A and colchicine which disrupt lymphocyte microfilaments and microtubules were used in studies by Anderson *et al.* (1978a, b, 1979) to show that emigration was dependent upon a functional cytoskeleton (Fig. 9). Those studies provided additional information. Cytochalasin A (CA) clearly blocked lymphocyte locomotion without preventing homing. Despite the absence of microvilli, some of these treated lymphocytes recognized and attached to HEV surfaces via membrane segments associated with clusters of microfilaments which remained anchored to the inner leaflet of the membrane.

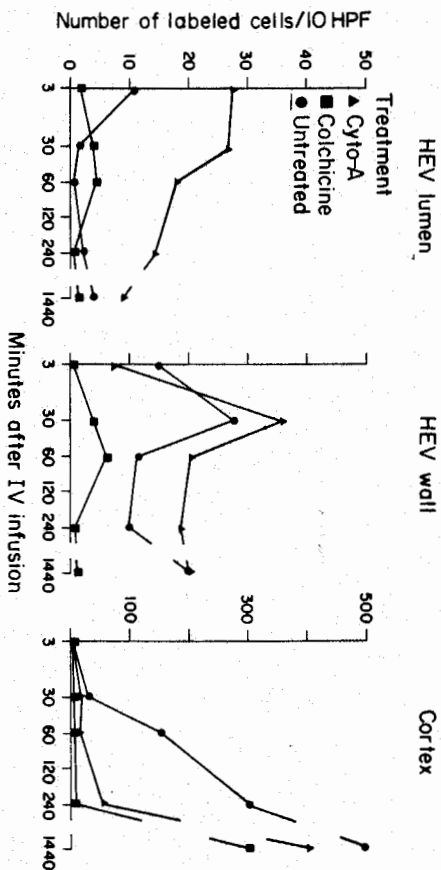


FIG. 10 Autoradiography of lymphocyte entry into lymph nodes after intravenous infusion was quantitated. Untreated TDL were rapidly cleared from the surrounding parenchyma in a nearly linear fashion. Lymphocytes treated with $4.0 \mu\text{g ml}^{-1}$ CA persisted in significant numbers along luminal surfaces of HEV. Increases in interendothelial cells were also seen, and a small percentage of the CA-treated TDL gained access to the lymph node cortex. Lymphocytes treated with 10^{-4} M colchicine showed reduced luminal accumulation at 3 min post-infusion, and remained in the circulation.

Residual motility in cells treated with intermediate doses of CA resulted in eccentric and markedly distorted emigration morphologies by cells which bore similar aggregated filament-rich adhesion sites. Colchicine treatment resulted in accumulation of treated cells in the circulating blood, and these cells failed to stick to HEV surfaces in radioautographic studies (Fig. 10). Other studies of the effects of metabolic inhibitors and agents which activate cytoplasmic cyclic AMP lymphocyte homing and recirculation *in vivo* tended to support the above observations (Smith and Ford, 1979; Freitas and Bognacki, 1979).

C. GENETIC FACTORS IN LYMPHOCYTE HOMING AND RECIRCULATION

Genetic identity has long been known to be a basic requirement for successful cellular transfer of immunity (Landsteiner and Chase, 1942). This is apparently due to the requirement of identity of major histocompatibility gene-determined surface receptors for immune cells to cooperate for the production of antibody or cellular cytotoxicity (Katz, 1977). The early studies of lymphocyte recirculation were performed in inbred species as insurance against introducing artifacts caused by genetic differences, for examples, graft-versus-host or host-versus-graft phenomena. As a result of this precedent, almost all studies of lymphocyte recirculation have been performed using autologous lymphocytes or lymphocytes collected from syngeneic donors. A few recent studies compared the homing capacity of syngeneic and allogeneic lymphocytes and concluded that homing was impaired in allogeneic combinations (Heslop and Hardy, 1971). Later studies in mice (Viklicky *et al.*, 1976) and sheep (Frost *et al.*, 1975) provided additional evidence that products of the major histocompatibility complex may be involved in lymphocyte homing to lymph nodes. Most recently, non-identity of both H-2K and H-2D determinants in mice were shown to significantly impair homing and localization of labeled lymphocytes in lymph nodes after intravenous infusion (Degos *et al.*, 1979; Viklicky and Micková, 1979). In these genetic studies, congenic mice with identical H-2 determinants but different back-grounds and recombinant strains carrying different H-2 haplotypes on the same B10 background were used to obtain controlled, quantitative data about the genetic determinants controlling lymphocyte homing and accumulation in lymph nodes. They found that complete H-2 identity, or at least identity of the K and/or D region genes, would result in optimal homing regardless of whether these haplotypes were on the same or different backgrounds. I-region identity did not seem to

be relevant unless it was in combination with at least a K or D identity. A problem in interpreting these observations is whether decreased lymph node homing resulted from the failure of allogeneic or semi-allogeneic lymphocytes to recognize lymph node homing sites or from elimination of these cells from the blood circulation by another mechanism, such as sequestration on RE cells in the liver or spleen. Because of the relatively small uptake of labeled lymphocytes in lymph nodes (12% of the injected dose in 24 h) compared to the combined uptake in the liver and spleen (30-35% of injected dose), a minor incremental increase in RE organ uptake would result in major deprivation of circulating cells available to enter lymph nodes. Degos *et al.* (1979) admitted that there was a large splenic and hepatic uptake, but did not show the data. Since T cell antigen receptors with specificity for foreign MHC determinants have been demonstrated in the serum of rats which have never been immunized (Binz and Wiggell, 1975a, b, 1976; Binz *et al.*, 1979), it is possible that a cellular immune phenomenon analogous to the humoral ABO blood group system may exist. If this is true, then the MHC non-identical lymphoid cells would bind the circulating T cell alloantigen receptor causing them to alter their homing characteristics either because their MHC-determined homing receptors are masked or because the homing receptor/T cell alloantigen receptor complex causes a new affinity for Ia-bearing RE cells in the liver and spleen. Feldmann (1973) proposed a mechanism for immune cell cooperation, where antigen-specific T cell receptor (he called it Ig-T) bound to macrophage surfaces after complexing with antigen and release from the T cell surface. In light of current knowledge of the T cell receptor for antigen, Feldmann's Ig-T, which had affinity for macrophage surfaces is identical to the T cell receptor removed from normal rat serum by anti-idiotypic immunoadsorbent columns (Binz *et al.*, 1979). There is also a possibility that preformed T cell alloantigen receptor might be directly injurious or might activate natural killer cell aggression toward the foreign cells, as indicated by data showing increased urinary excretion of ⁵¹Cr-label in recipients of labeled allogeneic cells (McNeillage and Heslop, 1980). However, simple or multiple minor H-2 disparities (which could be sufficient to cause graft rejection) did not prevent homing as long as one or both K or D regions were identical. The K and D regions apparently code for surface molecules, which are important for antitumor or antiviral cellular cytotoxicity, where adhesion to the target cell is necessary for killing (Zinkernagel and Doherty, 1975). It may be that K and D region-determined molecules regulate high avidity cellular adhesion, and non-K- or non-D-determined molecules

affect adhesion so minimally that differences in these genes are inconsequential. Studies of specific positive and specific negative selection of H-2 reactive cells (Wilson *et al.*, 1976) have taken advantage of the observation that allogeneic and/or alloreactive cells can be deleted from the recirculating pool by filtration through a recipient of the appropriate genetic composition (Howard and Wilson, 1974; Sprent and Miller, 1976). However, allogeneic and xenological lymphocytes show normal patterns of recirculation in thymus-less nude mice lacking auto-antiallotypic T cell receptors. These data suggest that MHC surface antigenic identity is a requirement for unimpeded recirculation in normal animals, but the defect in lymph node homing described by Degos *et al.* (1979) was due to depletion of homing cells from the circulation and not failure of these cells to recognize migration sites.

IV. Migration and differentiation of lymphocytes during development

Studies of the ontogeny of the immune system in rats and other animal models indicate that lymphocyte diversity results from proliferation and differentiation of precursor cells within various developing tissues; ultimately this differentiation continues during extraterine life within the thymus, bone marrow and peripheral lymphatic tissues. The resultant immunocompetent cells are committed to a particular cell lineage and effector or regulator function which can be established by the phenotypic markers expressed on their membranes. Rat models are useful in studying the end-stages of ontogeny, since the development and population of peripheral lymphatic tissues occurs during the first three or four days after birth. The intrauterine development of primary lymphoid organs and populations of lymphocytes in rat fetuses parallels in much shorter time intervals the development of human lymphoid tissue, as indicated by the tissue studies of Bailey *et al.* (1975a, b).

A. T CELL DIFFERENTIATION AND MATURATION

In rat embryos pluripotential stem cells for erythrocytes and leukocytes are present in the yolk sac early in gestation. The stem cells for lymphocytes resemble large blast cells, but lack immunocompetence and typical lymphocyte surface markers. These cells migrate into hepatic sinusoids and produce lymphoid, myeloid and erythroid colonies. The liver remains the major site of fetal hemopoiesis until shortly before birth. Since venous blood returning from the placenta

flows through the liver before being mixed with the circulating fetal blood, it is likely that developing lymphoid cells are exposed to maternal antigens, cells and antibodies which may have passed the placental barrier. The liver may thus be functioning as a bursal-equivalent, since the bursa of Fabricius also develops from yolk sac tissue, which is privy to environmental antigens crossing the shell of developing fowl eggs. Colonies of T cell precursors are programmed to display some T cell characteristics even before thymic development begins, since fetal liver cells stimulated by allogeneic lymphocytes will proliferate and express T cell surface markers.

The thymus develops from endoderm derived from the third and part of the fourth branchial pouches and its epithelial cells differentiate under the inductive stimuli provided by mesenchymal components. Primitive T lymphocytes infiltrate the gland by wedging themselves between adjacent epithelial cells. Proliferation and entry of new precursors distend the interepithelial spaces, resulting in densely packed clusters of lymphoid cells surrounded by an epithelial cell reticulum linked together by desmosomes. Macrophages and macrophage-like cells also infiltrate the thymus; recent *in vitro* studies indicate that factors released by these cells also influence thymic cell maturation (Beller and Unanue, 1978; Oppenheim, 1981).

Following birth and continuing until long after sexual maturity, stem cells originating in the bone marrow, move through the blood stream, pass through the walls of blood vessels in the outer cortex of the thymus, and infiltrate the epithelial reticulum. These cells proliferate rapidly and exhibit population turnover times of 24-36 h. Many of these lymphocytes may die *in situ*, but they are endocytosed and degraded by parenchymal macrophages so quickly that morphological evidence of cell death in normal thymuses is difficult to demonstrate by light microscopy. The surviving cells leave the thymus by migrating across the corticomedullary junction and exit to the blood via post-capillary venules or pass directly into efferent lymphatics in interlobular septae. The emigrant cells then pass into the blood and seed peripheral lymphatic tissues, where they carry out the various T cell functions in immune responses. Recent studies indicate that short-lived thymic cortical cells preferentially accumulate in the spleen, while long-lived thymic medullary cells recirculate and accumulate in lymph nodes. Studies of cell-surface differentiation markers in mice indicate that the cells bearing these proteins have important functional differences. In mice, some marrow stem cells exhibit faint staining for the T cell specific Thy-1 (theta) antigen. These cells appear to be prepared for T cell differentiation, since

mitogens or thymic factors can induce them to display the surface markers of mature T cells *in vitro*. The surface marker changes of these cells as they infiltrate the thymus include: (a) loss of surface TL (thymic-lymphoma) antigen; (b) secretion of addition Thy-1 surface antigens; and (c) acquisition of new differentiation antigens from the Ly system described by Cantor and Boyse (1972), which define additional functions of T cell subpopulations. About 50% of peripheral blood thymocytes express all three Ly antigens [Ly^(1+2+.3+)]. These cells may be precursor cells which will differentiate into specific accessory T cells after exposure to antigens and/or help from T cells. T cells expressing the Ly⁽¹⁺⁾ determinant are long-lived and recirculate and function as helper cells for both humoral and cellular immune responses. The subpopulation of T cells expressing Ly^(2+.3+) determinants function as suppressor cells and/or mediate cellular cytotoxicity against foreign target cells. Ly^(1+2+.3+) cells appear to be short-lived, since they rapidly disappear from the circulation after adult thymectomy; Ly⁽¹⁺⁾ and Ly^(2+.3+) cells persist for relatively long periods.

Early studies indicated that the primary function of the thymus was to confer immunocompetence upon developing and peripheralized T cells (Miller, 1975); relatively recent studies suggest that this function is equally shared with the more important service of maintaining immunological tolerance to self-antigens and beneficial molecules which must be able to persist in the body, such as food substances en route to centers of bodily assimilation. This may be underscored by the relatively heavy traffic of Ly^(2+.3+) suppressor cells to the Peyer's patches and perifollicular mantels of lymph node germinal follicles described by de Sousa *et al.* (1979).

B. B CELL DIFFERENTIATION AND MATURATION

The germ-line genes presumably carry the full complement of genes required for making every possible antibody, but due to random selection processes during early maturation each primordial B cell expresses only the very limited portion of this information required to react with a single antigenic determinant. B cell differentiation and proliferation begin in the liver and continue in the bone marrow as maturation continues. These lymphocytes contain cytoplasmic immunoglobulin, but for unknown reasons do not express this protein on their surfaces. Shortly before birth, lymphocytes with detectable surface IgM are present in bone marrow and this is followed by the appearance of other cells bearing IgD or both IgD and IgM on their

surfaces. Thereafter, B cells bearing all the mature immunoglobulin isotypes can be detected and these cells proliferate and populate lymphatic tissues without maturing into plasma cells. The B cell system appears to be more susceptible to tolerance induction in the early phases of neonatal differentiation (Nossal, 1979). Moreover, the injection of antibodies directed against isotypic, allotypic or idiotypic immunoglobulin determinants can lead to long-lasting inactivation of antigen-reactivity of B cells bearing these proteins on their membranes. This tolerant state is readily produced when the maturing B cells express only IgM, and is more difficult to achieve after the IgD marker is expressed. Further, anti-immunoglobulin can induce idiotype-specific T-suppressor cells which may induce tolerance of another sort.

Studies of the life-span of T and B lymphocytes (Sprent and Basten, 1973; Sprent, 1977) suggest that B cells live shorter lives than T cells. This may actually be an artifact of the radiolabel methods used to quantitate the life span. B cells, or their progeny, may live on after antigen-induced proliferation which breaks the train and scores as a loss of ³H-thymidine-bearing interphase B cells. If the life span of the average B cell is actually shorter, then a greater degree of antigen-independent proliferation must be present in order to maintain adequate numbers of clonal precursor cells to react with the 10⁶-10⁷ various antigenic specificities. The bone marrow most obviously serves this purpose; but is it also possible that germinal follicles dispersed among all the lymphoid tissues of the body might also function to turn out precursor progeny. Recent evidence suggests that this might be the case; but, environmental antigens may be the stimulus for the continued production of precursor B cells which require an additional hit by antigen and/or accessory cell factors for final proliferation, differentiation and maturation into antibody-secreting plasma cells (Cebra *et al.*, 1977).

C. DEVELOPING LYMPHATIC TISSUES

The peripheral lymphatic tissues of the rat complete their structural development and are populated by lymphocytes during the first few days of extrauterine life. Since certain microenvironments in these peripheral tissues apparently depend upon the cellular or hormonal contributions of the thymus, it is possible to impede the development of these regions in newborn rats and mice by removing the thymus immediately after birth (Parrott *et al.*, 1966). However, other structures in lymphatic tissues develop normally in the absence of a

thymus, but thymus engraftment or infusion of thymic lymphocytes restores the total tissue, thereby indicating that local mesenchymal structures are also vitally important in the formation of lymphatic tissue microenvironments.

1. Lymph node

The earliest lymph node to develop in the newborn rat is the mesenteric. Immediately after birth this node is a lymphatic sac lying near the neurovascular bundle in the mesentery. Reticular cells are present, but the sac is not yet partitioned into lymphatic sinusoidal spaces and reticulum. Development of lymphatic channels occurs first; by the second day a subcapsular sinus and some medullary sinuses appear. Lymphocytes are present in the peripheral blood, but none have entered the lymph nodes. Between days 2 and 3 the small venous vessels in the developing lymph nodes undergo proliferation and segments of vessel appear, which have plump endothelial cells capable of reacting positively in nonspecific esterase histochemical preparations. Concomitant with the development of these new vessels, small lymphocytes begin to populate the mesenteric lymph node. Diffuse lymphoid tissue lacking germinal follicles and plasma cell cords is present by day 7; by 14 days the lymph node appears almost fully formed. A network of nonbranching HEV can be seen deployed vertically between the subcapsular sinus and the hilum in lymph nodes perfused intraarterially with alcian blue dye (Fig. 11). By 12 weeks of age, most of the lymph nodes in the rat contain diffuse collections of lymphocytes, cords of plasma cells and germinal follicles.

2. Spleen

The architecture of the rat spleen is apparently more developed than the lymph node at birth. The red pulp reticulum is a functional RE filter and also maintains some extramedullary hematopoiesis. The white pulp is fairly well developed. The periarteriolar lymphatic sheath (PALS) is delimited from the red pulp by a marginal zone rich

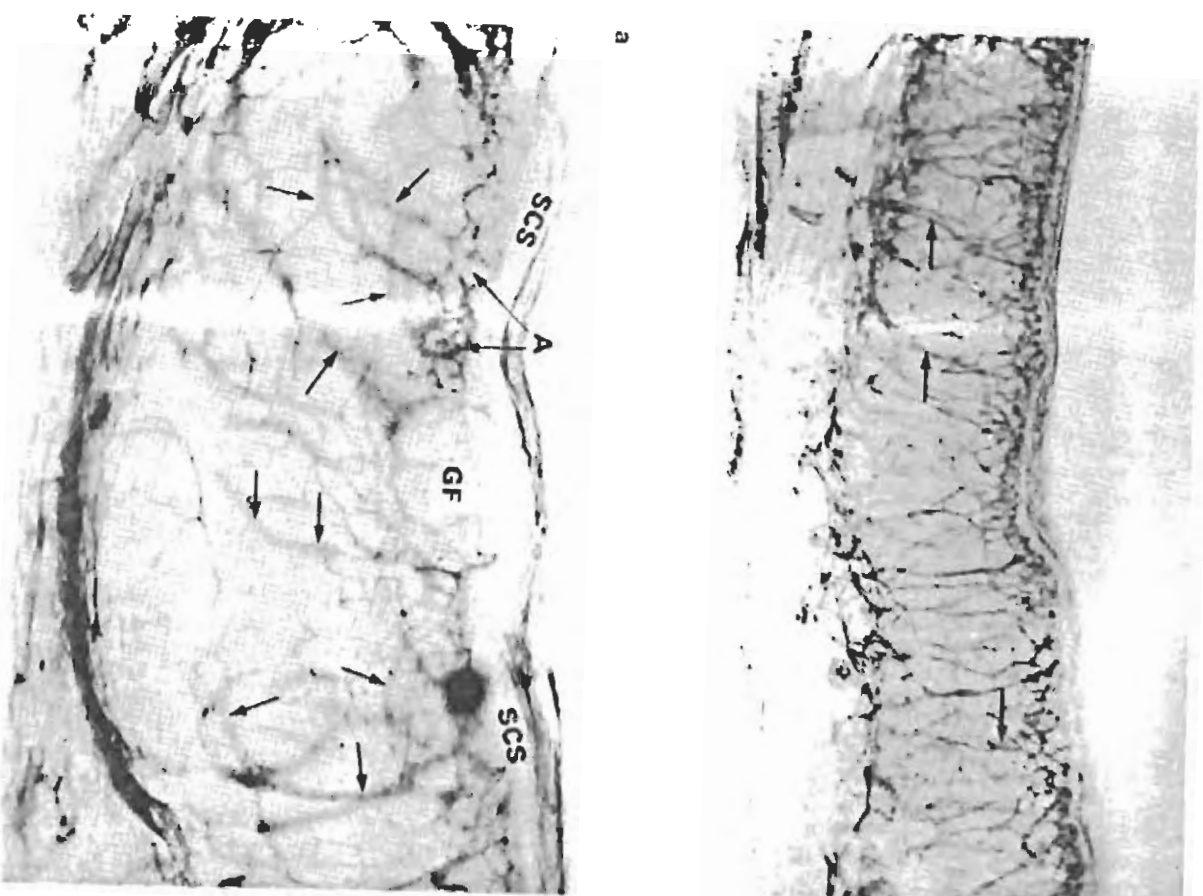


FIG. 11 These are alcian blue dye perfusion preparations of lymph nodes from 7-day old (a) and 84-day old (b) rats. Note the relatively simple arrangement of blood vessels in (a) compared to (b). The adult rat lymph node has networks of HEV (arrows); germinal follicles (GF) interrupt the cortical vascular arcades (A) and the subcapsular lymphatic sinus (SCS).

in esterase-positive monocyctic cells. There are clusters of small lymphocytes in the periaerterolar lymphatic sheath; some cells can be seen lining up in what appear to be networks of lymphatic capillaries which bridge PALS regions in a "chicken wire" network. Retrograde infusions of India ink from the thoracic duct to the spleen parenchyma reveal these lymphatics (Fig. 12). The newborn rat spleen appears to be completely organized, with respect to the partitioning of lymphoid and red pulp tissues, by the end of the first week of extrauterine life.

5. Peyer's patches

Peyer's patches do not appear to be morphologically recognizable in the intestines of late fetal and newborn rats. However, Parrot (1976) has shown that an environment exists which subsequently becomes populated with lymphoid cells when these loops of intestine are grafted beneath the renal capsules of mature syngeneic rats. In addition, unpublished studies of Anderson and Anderson (1974) indicate that an epithelial and connective tissue site which is predestined to become a Peyer's patch exists in these immature rats. By retrograde infusion of

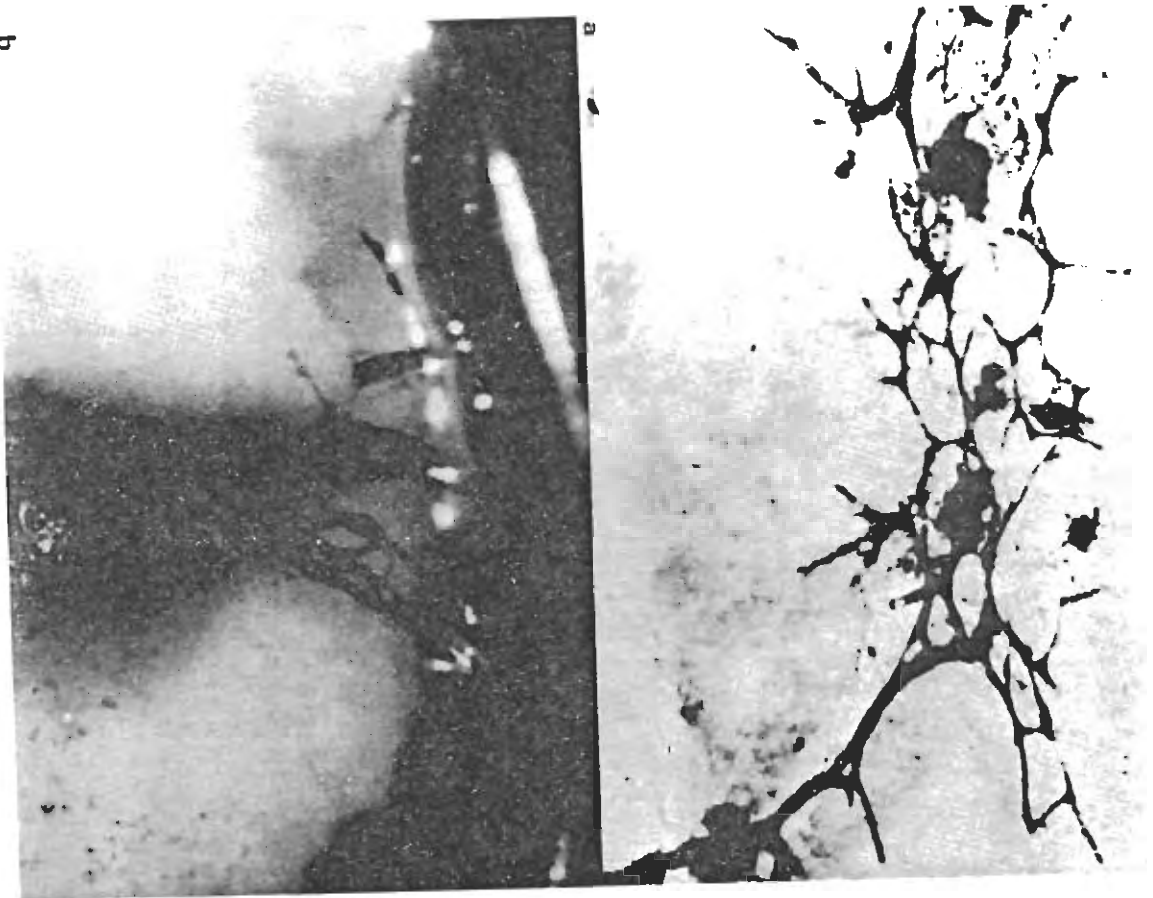


FIG. 12 Retrograde perfusion of the thoracic duct with India ink delineates the network of lymphatic channels present within (a) and draining from (b) the newborn rat spleen. These lymphatic channels connect PALS regions through anastomoses which are perpendicular to the PALS, similar to that described for the bridging zones of Mitchell (1973).

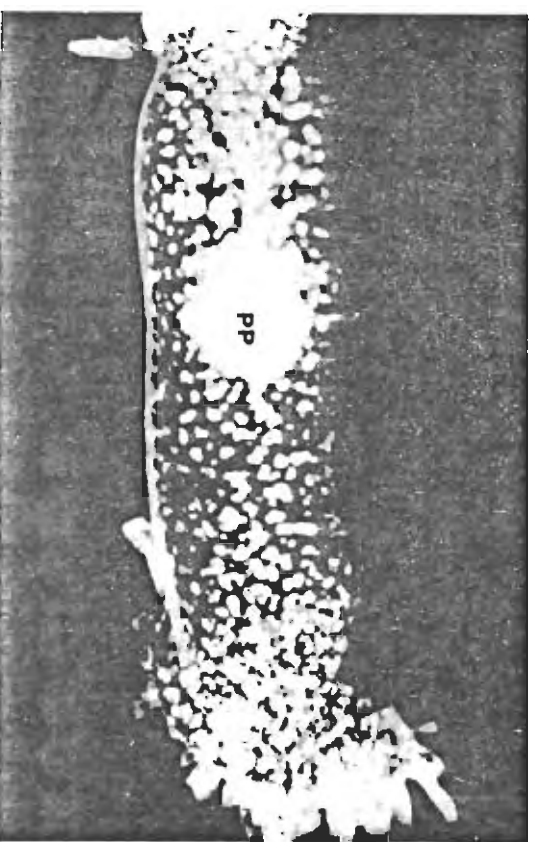


FIG. 13 The connective tissue stroma of the developing Peyer's patch (PP) excludes collateral lymph flow from the submucosal and subserosal lymphatic plexuses. When these spots are examined by electron microscopy, they are devoid of lymphocytes, but possess reticular meshworks and an overlying epithelium which is very endocytic.

India ink into the mesenteric lymphatics, nonstaining patches on the antimesenteric border of the intestine can be discerned because the ink blackens the subserosal lymphatic network up to, but not through, these sites (Fig. 13). Plastic, 1-mm thick sections and ultrastructural examination of these patches reveals that they lack germinal follicles and small lymphocytes, but contain reticular cells, lymphatic channels and an overlying epithelium, which exhibits extensive endocytic activity. Again, small lymphocytes do not begin to populate Peyer's patches until extraterine day 2, when esterase-positive HEV develop. Germinal follicles begin to appear in Peyer's patches by days 14 to 28, which is paralleled by the population of the interfollicular areas with recirculating T cells.

V. Lymphatic tissues in the adult rat

The lymphatic tissues in adult rats may be grouped into central and peripheral lymphoid organs, as are found in most mammalian species. The central lymphoid organs, consisting of the bone marrow and the thymus, are where antigen-independent proliferation and differentiation of B and T lymphocytes continue into adult life. The peripheral lymphatic tissues include the lymph nodes, spleen and submucosal lymphoid nodules of the respiratory and gastrointestinal systems. While some antigen-independent lymphocyte development might occur in peripheral lymphoid tissue, most of this activity is usually related to antigen exposure.

A. CENTRAL LYMPHATIC TISSUES

1. Bone marrow

The bone marrow serves as a protected environment where lymphocyte precursors undergo the antigen-independent proliferative steps and genetic recombinations necessary for the continued generation of diverse repertoires of immune cells. The bone marrow's aggregate mass is about equal to that of the liver. It is a vascular organ composed of central and radiating venous sinuses which surround and separate wedge-shaped interstitial compartments containing hematopoietic cells. These compartments are separated from the vascular spaces by a thin endothelium, which forms an irregular sandwich with the adventitial cells of the interstitium. The granular basement membrane between endothelium and adventitia shares many morphological characteristics with reticular fibers found in peripheral lymphatic

tissues. The adventitial cells face the hematopoietic areas and extend cytoplasmic processes into the cellular compartment. The white cell components generated within the interstitium traverse the sinus endothelium by actively migrating between endothelial and adventitial cells. Since the sinus endothelium is very attenuated, intravasating leukocytes might pass through parts of the endothelial cell cytoplasm, but this point is controversial. The sinus lining-cell complex is thought to bear some regulatory influence upon circulating leukocyte levels by controlling which cells enter the blood (Weiss, 1972). Apparently, only cells of sufficient maturity are capable of crossing this vascular barrier.

Lymphocytes and their precursors are usually scattered among the other hematopoietic cells, but occasionally are found in discrete nodules. Up to 20% of the nucleated cells in marrow may be lymphoid cells, but in hyperimmunized animals or following corticosteroid treatment, the marrow concentration of small lymphocytes is much higher. Macrophages are frequently found among clusters of rapidly dividing lymphocytes. These cells may encourage continued proliferation by removing effete or aberrant lymphocytes, and releasing recycled proteins and nucleic acids along with lymphocyte-activating factors (LAF).

2. Thymus

The thymus is a pale, lobulated organ which is largest, relative to total body weight, at birth. It gradually increases in size until sexual maturity, after which it involutes slowly. Each lobule is composed of a cortex and medulla. The densely populated cortex forms the periphery, while the more sparsely populated medulla branches to form the central portion of each lobule. Inside the cortex, large numbers of rapidly dividing lymphocytes form multicellular nests which stretch and distort the desmosome-linked epithelial cells into a honey-combed reticulum. These epithelial cells display major histocompatibility antigens, including Ia, H-2K and H-2D (Rouse *et al.*, 1979). The presence of these "self" antigens within the thymus may be an underlying microenvironmental feature responsible for self-tolerance and nonself-recognition by T cells.

a. Special cells in the thymus

At the corticomedullary junction, a thin basal lamina and specialized mesenchymal cells separate the cortical from the medullary lymphoid populations. Macrophages and interdigitating cells which resemble Langerhans' cells (Oláh *et al.*, 1975) are found within the epithelial

of H-2 type restriction (Zinkernagel & Doherty, 1974) and apparently to antigen presentation.

stroma of the thymus. The macrophages contain large phagolysosomes filled with nuclear debris and periodic acid Schiff-positive membrane glycoproteins. These macrophages may also exert a stimulatory or differentiating effect on surrounding thymocytes by releasing LAF or other factors (Beller and Unanue, 1978). Thymic "nurse" cells derived from the epithelial reticular cells have also been isolated *in vitro* (Wekerle *et al.*, 1980). These large cells appear to encase clusters of proliferating and differentiating lymphocytes. Since they display Ia antigens, they may serve an instructive role in self-tolerance or self-recognition. "Special cells", with distinctive electron-lucent cytoplasm, striated cytoplasmic granules and multiple interdigitating extensions and processes, have been found at the corticomedullary border. These cells resemble novel dendritic cells of peripheral lymphatic tissues (Steinman *et al.*, 1975; Steinman and Witmer, 1978) and Ia antigen-bearing Langerhans' cells (Silberberg-Sinakin *et al.*, 1976; Stingl *et al.*, 1978), which have been shown to be effective presenters of skin-sensitizing antigens to T cells. Whether these corticomedullary "special cells" present antigen or tolerogen to maturing T cells, which migrate *past en route* to the medulla and the circulation, remains to be shown.

There are fewer lymphoid cells in the thymic medulla, which causes the epithelial cells to appear more polygonal and glandular. Some of these cells form concentric whorls and become keratinized. These unusual structures are called Hassel's corpuscles. Their function remains obscure, despite their distinctive appearance. Some morphologists feel that these structures provide for the destruction and turnover of epithelial reticular cells, since the centers of the Hassel's corpuscles frequently contain acid hydrolases.

b. *The blood-thymus barrier*

A blood-thymus barrier was initially proposed because of differences in immune responsiveness to antigens injected directly into the thymus versus intravenously (Marshall and White, 1961). Since intrathymic injection of antigen resulted in local development of germinal follicles and other structures associated with B cell immune responses, the differences in immune responsiveness observed after immunization by these routes could be attributed to changes in the thymic micro-environment, rather than a blood-thymus barrier. Horuchi and Waksman (1968) used a different antigen, but a similar experimental design and found that intrathymic inoculation with antigen resulted in the induction of tolerance. This apparently contradictory result lead to reexamination of the blood-thymus barrier, since systemic antigens may produce subtle effects on thymus lymphoid populations.

A number of anatomical studies, which specifically addressed the question of blood vessel permeability within the thymus, showed that cortical thymocytes, in contrast to those of the medulla, are protected from circulating macromolecules (Chapman and Bopp, 1970; Ravioia and Karnovsky, 1972). Blood vessels (Fig. 14) in the thymic cortex were

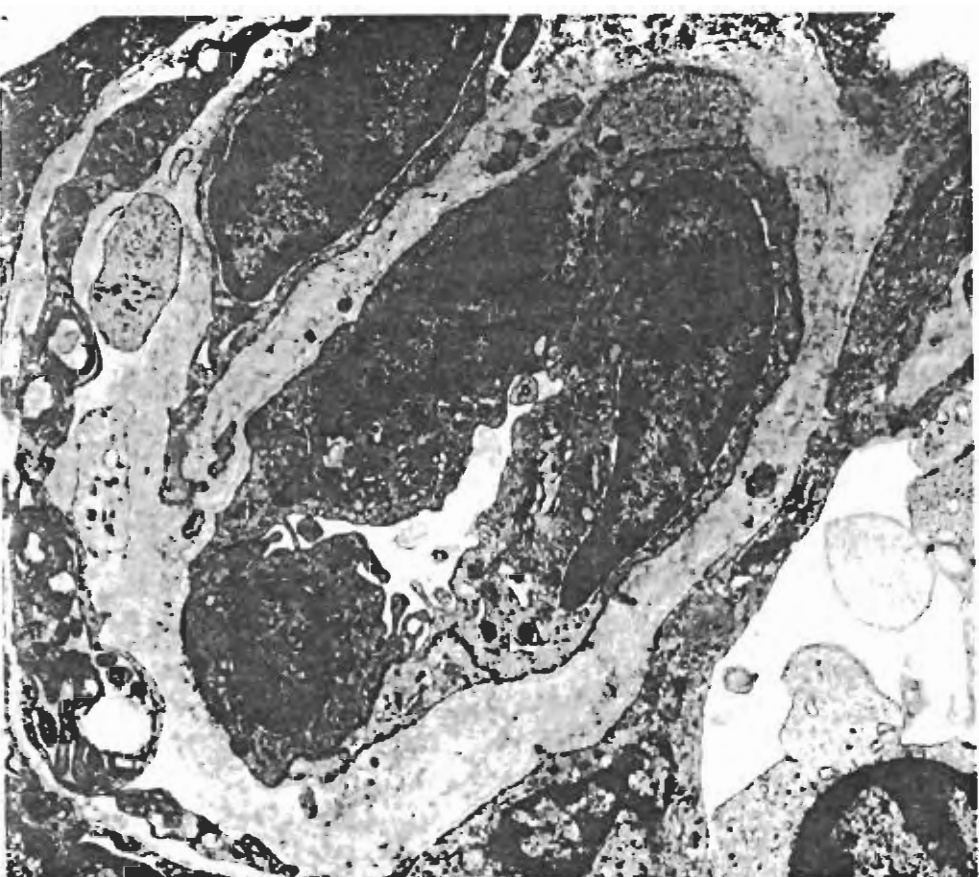


FIG. 14 This is a blood vessel located in the cortex of the thymus. Note the thick basement membrane and complex perivascular sheath.

not permeable to the physiologic tracers, but the postcapillary venules of the thymic medulla permitted tracers to leak along the clefts between migrating lymphocytes and endothelial cells. The tracers which crossed medullary venule walls had limited distribution in the thymic parenchyma because macrophages in the perivascular space ingest and retain much of the leaked tracer. The tissue distribution of the small amounts of antigenic tracer which escaped endocytosis was further limited to the medulla by the phagocytic cells and basal lamina at the corticomedullary border.

B. PERIPHERAL LYMPHOID ORGANS

The peripheral lymphoid organs are encapsulated and nonencapsulated reticular structures which provide sites for the interaction of immune cells with antigen by way of their afferent lymph and blood vascular connections and their constant access to circulating immunocompetent cells. Variations in the structure and location of these organized lymphatic tissues appear to represent specific adaptations, which assist the host in mobilizing immune responses against pathogens invading distant tissue sites, the blood stream and mucous membranes.

1. Lymph nodes

Lymph nodes are complex filters which interrupt the current of afferent lymph draining specific regions of the body. The lymph is forced to percolate through a reticulum containing lymphoid cells before gaining access to more recognizable efferent lymphatic channels.

Reticular cell meshworks provide a microenvironment which is conducive to: antigen trapping, adhesion and locomotion of lymphocytes and monocytes and dynamic sorting and functional grouping of cells which participate in immune responses. Reticular cells display fibronectin on their surfaces, which may contribute to establishing a lymphatic microenvironment (Steman and Vaheri, 1978). Fibronectin is an important cell-surface glycoprotein which participates in such diverse processes as coagulation (Mosher, 1975), particle opsonization (Blumenstock *et al.*, 1978) and contact inhibition of proliferation and locomotion (Vaheri *et al.*, 1976). Reticular cells are said to be phagocytic, but they primarily provide anchoring sites for macrophages which do the bulk of the phagocytosis. These cells may selectively endocytose and recycle complex molecules secreted by

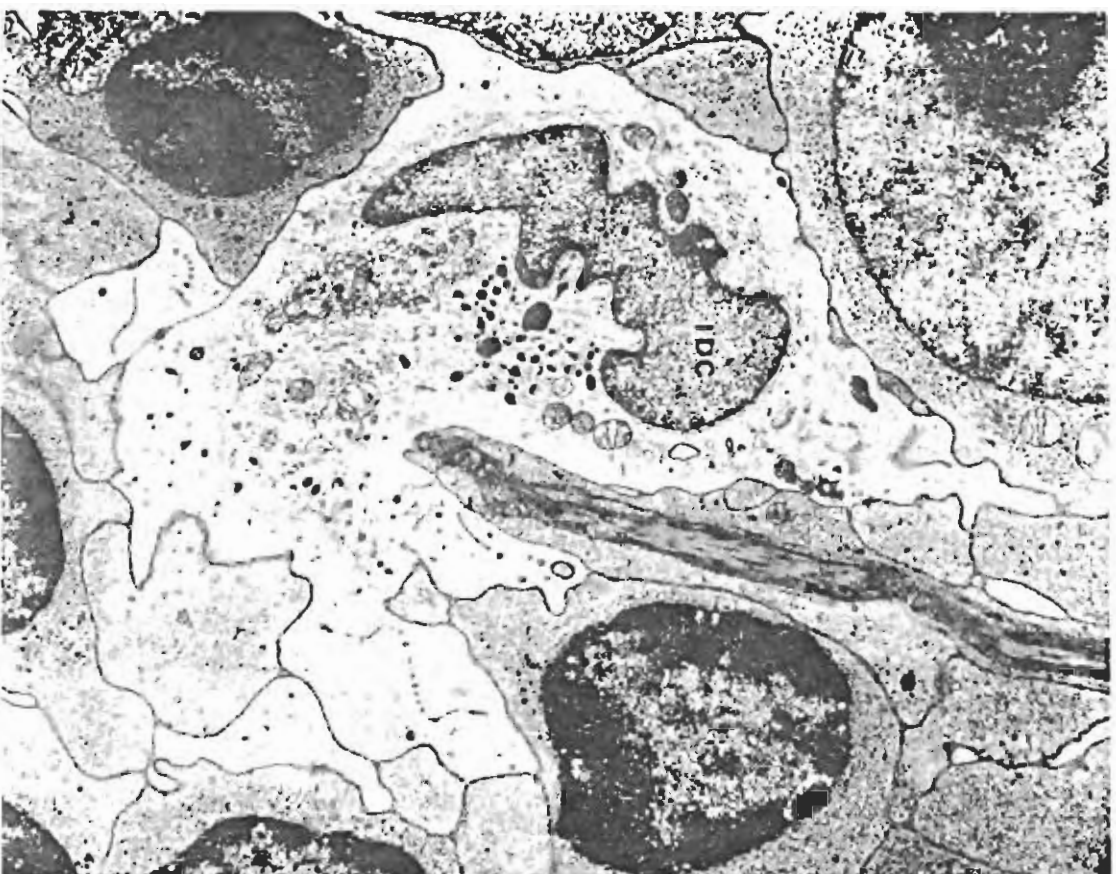
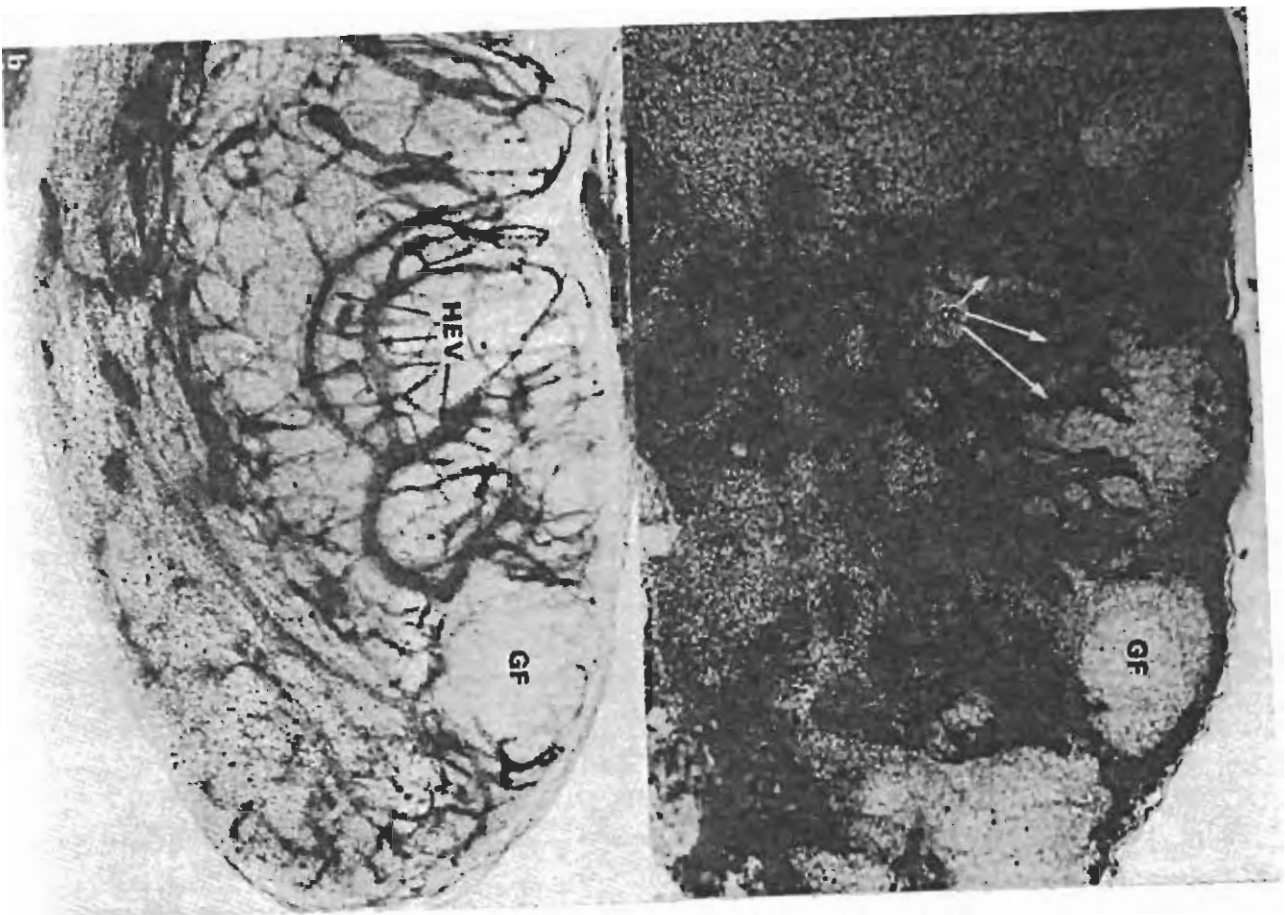


Fig. 15. This is an interdigitating (IDC), or novel dendritic cell (Steinman *et al.*, 1978) located in the paracortex of a rat lymph node. These cells are virtually identical in morphology to the "special cell" found in the corticomedullary junction of the thymus and is similar to descriptions of Langerhan's cells found in regional lymph nodes. They display Ia antigens on their surfaces and prime T cells for mixed lymphocyte reactivity.



neighboring cells. This is illustrated by the observation that reticular cells engulf the heparin-rich stroma of secreted mast-cell granules (Fig. 17). The antigen-trapping function of these cells is related to the ability of meshworks of them to cause turbulence in lymph flow, which impacts particles against the surfaces of reticular cells and sinus macrophages. In addition, immunofluorescence studies indicate that immunoglobulins and antigens bind nonspecifically to reticular cell surfaces, possibly by utilizing intermediary molecules, such as heparin, fibronectin, and serum complement components.

a. Passage of lymph through lymph nodes

Afferent lymphatic vessels originate in delicate plexuses beneath the epithelium of the skin, gut and urogenital tract and within the connective tissues of all organs. These capillaries merge into larger lymphatics which drain into lymph nodes (Yoffey and Courtee, 1956). Efferent lymph from regional nodes may drain into one or two additional nodes before flowing into the large lymphatics which join the thoracic duct (from the lower part of the body) or the right lymphatic duct (from the upper thorax and head). These major efferent ducts return lymph to the blood by emptying into the great veins at the base of the neck. The lymph nodes which are interspersed along this network of channels serve as filtering chambers and are constructed of a meshwork of reticular cells and fibers, macrophages and specialized blood vessels enclosed in a tough connective tissue sac. Since all lymph passes through at least one lymph node before returning to the blood, any environmental antigens, such as bacteria, viruses or virus proteins expressed on host cell membranes, protein antigens and tumor cells, will be trapped; under normal conditions these materials would not enter the blood. Any antigens which might escape lymph node entrapment will ultimately be removed from the blood by macrophages in the spleen, liver or bone marrow. Sophisticated tracer and EM studies were needed to show that subcapsular sinuses are connected to medullary sinuses by delicate plexuses of intermediary sinuses (Fig. 16), and by channels created by vertically

FIG. 16 (a) Lymph flowing into the subcapsular lymphatic sinuses of a lymph node via afferent lymphatics is broken up into anastomosing smaller networks of lymph channels as it passes through the paracortex. (b) These intermediary sinuses (S) surround HEV so that the lymph node interstitium between the blood and lymph interfaces need not be wider than 10 or 20 lymphocytes. This is vitally important to the immunological schema, since recently emigrated lymphocytes will lie in parenchyma, which is close to the antigen-carrying lymph stream. Note also that germinal follicles are neither highly vascular nor infiltrated by lymph.

oriented reticular meshworks, which communicate with the floor of the subcapsular sinus through pores (Fig. 18). Macrophages cling to reticular cell processes inside lymph sinusoidal spaces. Antigens entering a lymph node for the first time will be bound to the surfaces of portal-guarding macrophages which endocytose most of the antigen, but leave some remaining on their surfaces (Nossal and

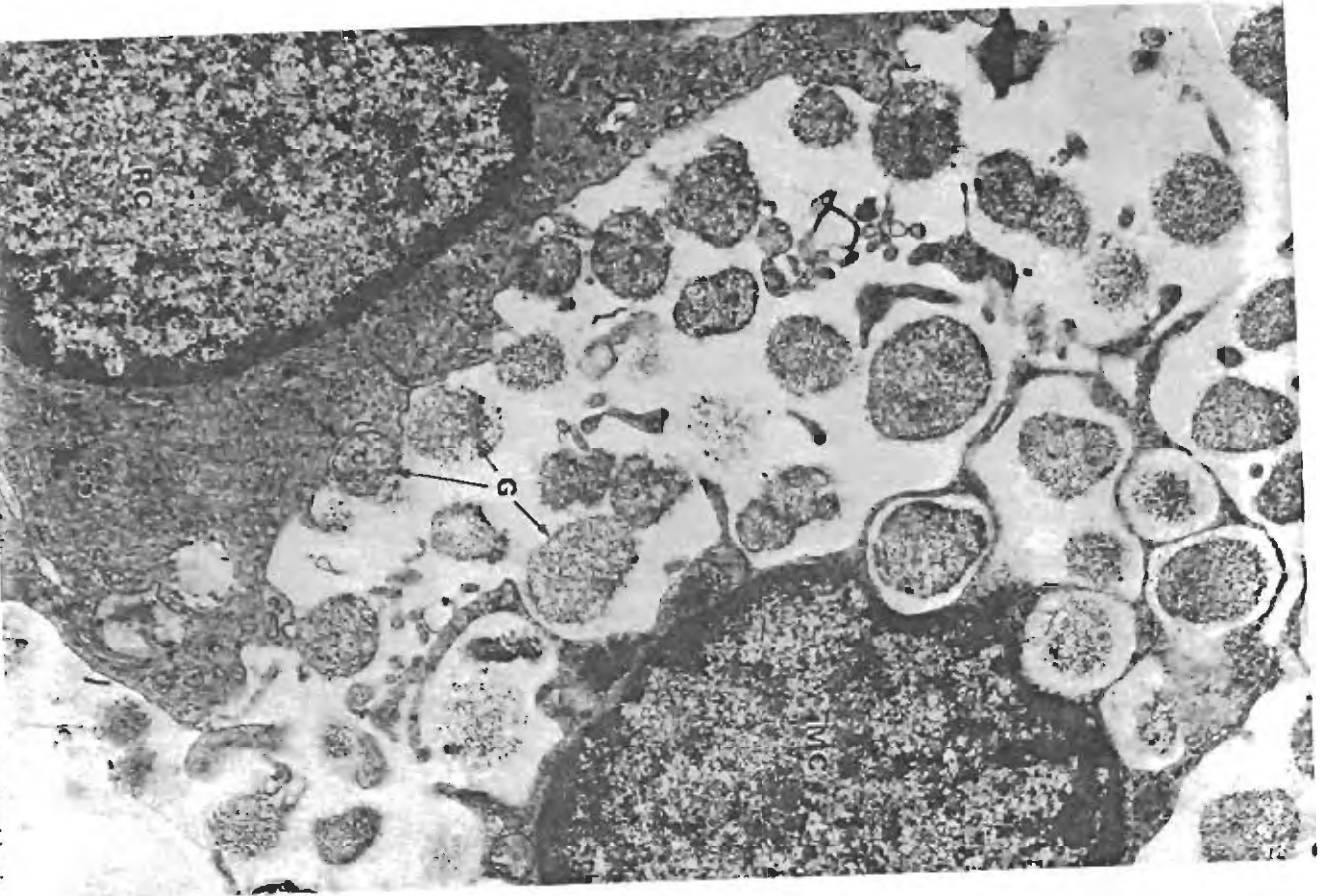


Fig. 17 Degranulating mast cells (MC) within lymph nodes interact with fibroblastic reticular cells (RC). The reticular cells endocytose the heparin-rich granule (G) stroma using "coated pits" (arrows).

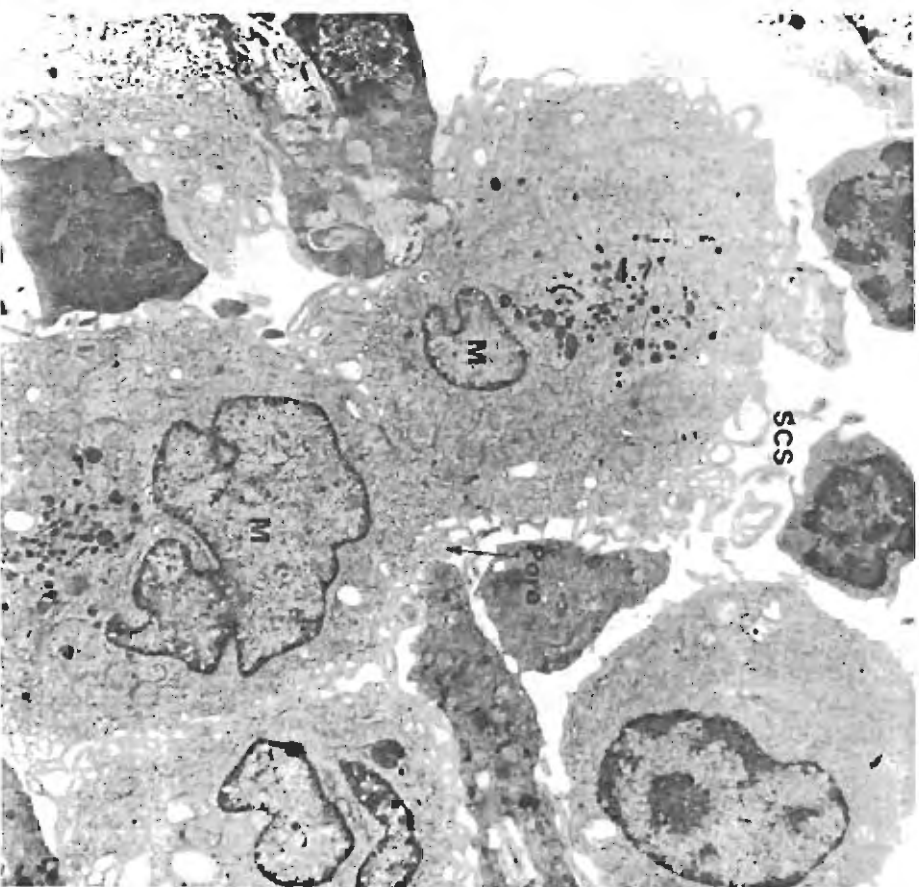


Fig. 18 Macrophages (M) guard every portal of lymphatic tissues. Here, two macrophages protrude through a pore in the floor of the lymph node subcapsular sinus (SCS).

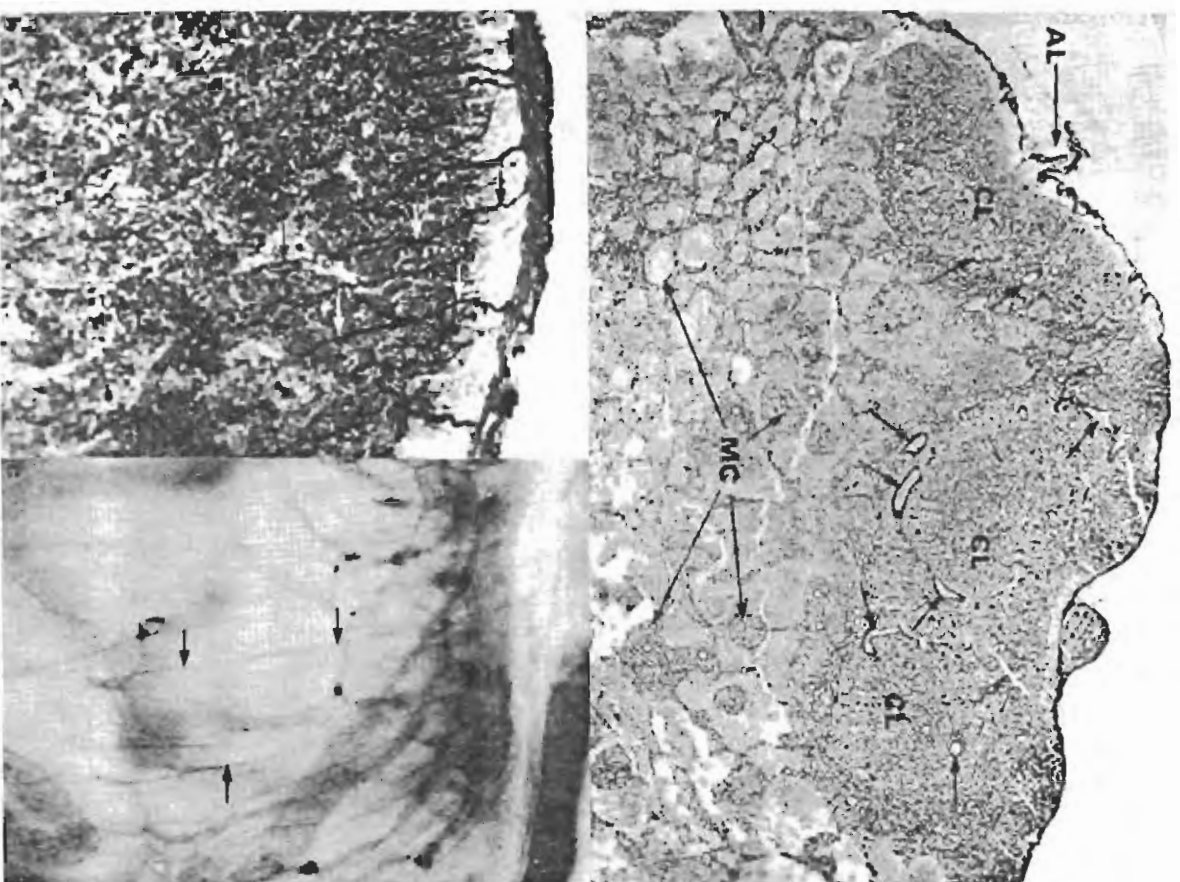


FIG. 19 The lobular architecture of the rat lymph node cortex is defined by the transitions in the organization of reticular cell meshworks at corticomedullary borders. (a) Silver impregnation methods reveal the dense meshwork of reticulum in cortical lobules (CL) and medullary cords (MC). Note how reticular fibers condense the

Ada, 1971). Reticular cells also bind antigen without phagocytosing. Langerhans' cells from epidermal skin sites (Stingl *et al.*, 1978) and/or novel interdigitating dendritic cells (Fig. 15) localize in the paracortex and serve an antigen-presenting role, which appears to affect T cells selectively. After 3 or 4 days, cytophilic antibody and complement facilitate binding of antigen by the dendritic cells within germinal follicles (Humphrey, 1976). Antigen remains bound to follicular dendritic cells for a fairly long period of time and the same antigen will bind to these cells with greater avidity on secondary exposure (Hanna and Szakal, 1968). The molecular mechanisms of antigen-binding during primary antigenic exposure may be related to natural antibody or to non-specific opsonins, such as fibronectin, displayed on macrophage and reticular cell surfaces or to absorption of inflammatory products, such as complement components, present in the antigen-containing afferent lymph (Pryjma and Humphrey, 1975). Antigen-binding after a secondary immune response is much more efficient, due to specific recognition provided by cytophilic antibodies bound to macrophage membranes at Fc receptors. The phagocytosis of antigens within the node may provide the antigen-processing and facilitate the macrophage helper functions for immune responses to thymus-dependent antigens.

b. Lymph node structure

Lymph nodes can be divided into cortex and medulla, based on the relative density of small lymphocytes. In large lymph nodes, cortical tissue is distributed as rounded lobules densely packed lymphocytes which are delimited on one side by the floor of the subcapsular sinus and on the other by the terminations of vertically oriented reticular fibers at the origins of medullary cords (Fig. 19). Germinal follicles in the superficial cortex partially interrupt the subcapsular sinus. These structures are generated by proliferating lymphocytes which displace the cortical reticulum to form a basket-like enclosure (Fig. 16). Inside the enclosure are lymphoblasts, intermediary lymphocytes, dendritic cells (Nossal *et al.*, 1968a, b) and "tingible-body" macrophages. On the outside is a mantle comprised almost entirely of small B

sheaths of HEV (arrows). (b) and (c) The parenchyma of cortical lobules is at a relatively uniform thickness (with respect to the subcapsular sinus) by vertically oriented fibers (arrows). In the rat, enlargement of the cortex induced by antigen usually results from lateral expansion of each lobule.

lymphocytes. Immunofluorescence (Weissman, 1975) and antibody-coated erythrocytes (Dukor *et al.*, 1970) delineate the follicular mantle as a B cell zone. The lymphocytes within the intermodular areas and the deeper cortex are largely T cells (the thymus-dependent zone). Since both B and T cells enter the lymph node parenchyma by passing through the walls of specialized venules in the deep cortex, the thymus-dependent zone should be thought of as a transient-cell zone containing about the same proportions of T and B cells presented to it by the blood. In most cases, this is 75% T cells and 25% B cells, but antigenic stimulation (Gery *et al.*, 1977) or treatment with adjuvants (Anderson and Reynolds, 1979) can alter these ratios considerably by supplementing the normal cell traffic with additional T or B cells from the blood or from the injection site.

Beneath the cortex and filling all the spaces between cortical lobules of large lymph nodes is the medullary tissue composed of wide sinuses which surround cords of reticulum filled with antibody-forming B lymphocytes and plasma cells.

c. Lymph node vasculature

The circulatory system of the lymph node serves the special physiologic requirements of a tissue populated by transient cells with widely fluctuating metabolic activities which are capable of rapid changes in cellular traffic after antigenic stimulation. Recirculating cells depend on local blood flow, permeability and balances between vascular and tissue hydrostatic pressure for the capacity to settle onto surfaces and for the generation of diffusible chemotactic signals.

Arterial vessels enter lymph nodes at the hilum and branch once or twice as they cross into cortical lobules. These vessels give off capillary twigs and arteriovenous communications at arcades of capillaries in the superficial cortex and in medullary cords. Arteriovenous communications and short venules connect with vessels lined by large polygonal endothelial cells (Fig. 20(a)) called HEV.

The distal segments of HEV open into capacitance vessels with wide lumens and thin walls which drain segments of cortical lobules and merge to form lobular veins. At sites where these veins anastomose with each other and with the systemic circulation, muscular contractile structures are found which may serve to prevent dramatic intranodal circulatory changes from influencing the systemic blood flow (Fig. 20(b)).

Little information has been provided about vascular structures which regulate blood flow in regional lymphatic tissues. Generalizations about blood flow in lymph nodes were based on model systems

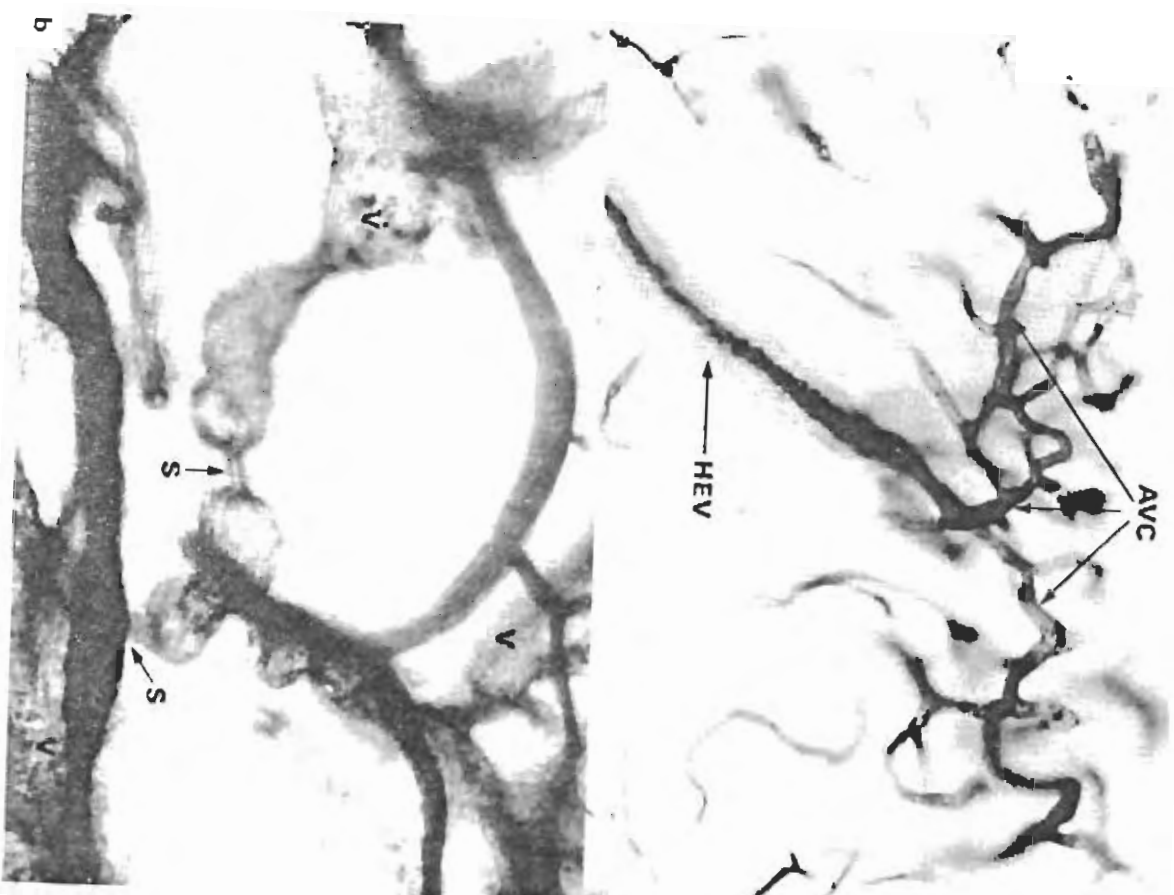


Fig. 20. These high magnification micrographs of an alcian blue preparation show the connections of arteriovenous communications (AVC) within the superficial cortex with a HEV (a) and venous sphincters (S) in lobular veins (V) located near the hilum (b).

where contraction of arterioles and precapillary sphincters regulated capillary flow (Calvert, 1897; Zimmerman, 1923; Schulze, 1925; Lundgren and Wallentin, 1964). Dabelow (1939) was the first to suggest that arteriovenous communications (AVC) were responsible for blood flow redistribution in lymph nodes, but he was unable to provide unequivocal anatomic demonstrations of arteriovenous anastomoses. Although some reports described AVC within mesenteric lymph nodes, these structures were not consistently found (Herman *et al.*, 1969;

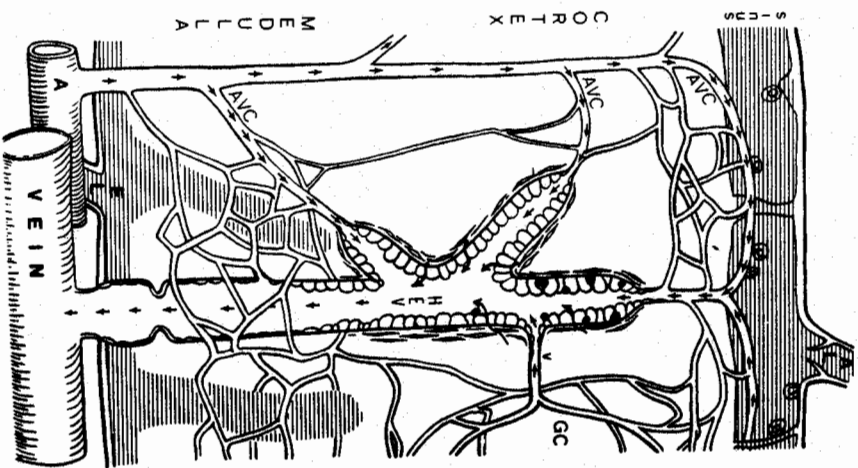


FIG. 21 The location of HEV between arteriovenous communications (AVC) and venous sphincters (VS) provides a unique system for regulating blood flow and pressure in lymph node venules. Afferent and efferent lymphatic connections are indicated by AL and EL and GC defines the displacement of AVC around germinal centers.

Davidson *et al.*, 1973). This difficulty in demonstrating AVC in routine perfusion preparations may be attributed to postexcisional constriction of the shunts (Baumel *et al.*, 1970). Direct intraarterial infusions with soluble alcian blue dye (Anderson and Anderson, 1975) demonstrated numerous AVC within the superficial cortex of axillary lymph nodes (Fig. 21). These vessels directly linked arterial circulation with HEV. Longitudinally deployed bundles of elastic tissue in the walls of the AVC permitted these vessels to remain intact during stretching and displacement by expanding germinal centers. The structure and distribution of AVC indicated that they participated in the shunting of blood past the cortical capillary arcades. Studies of regional blood flow through lymph nodes using double labeled 9- and 15- μ m microspheres clearly demonstrated normally elevated arteriovenous shunting along with total blood flow after antigenic stimulation (Herman *et al.*, 1978).

Little is known about the regulation of venous blood flow in lymph nodes. It is known from other systems that venous tone and segmented contraction or dilatation might influence efferent blood flow. In rat lymph nodes these types of control mechanisms would have to be located near the hilum, since HEV and lobular veins lack smooth muscle and their lumens are kept open by differences between vascular and tissue hydrostatic pressure. The existence of sphincteric structures, composed of circumferential bundles of smooth muscle, at terminal ends of thin-walled lobular veins suggested that the pressure and flow of efferent blood draining from individual cortical lobules could be regionally controlled. Lobular vein diameters ranging from 50 to 150 μ m in "stimulated" lymph nodes indicated wide variability in the normal tone of those sphincters in a given node. These and other observations suggested that lobular vein/venous sphincter complexes serve as capacitance/resistance structures which accommodate the wide volume and pressure changes which occur within lymph node vessels.

Studies of circulation changes in stimulated lymph nodes have demonstrated a biphasic shift in blood flow following antigen inoculation. An early increase in flow up to 30 times normal is associated with hyperemic effects induced by antigen (Hay and Hobbs, 1977). This increased flow is also associated with increased shunting (Herman *et al.*, 1979) and increased lymphocyte traffic rates (Ottoway and Parrott, 1979). The gradual return to increased blood flow which occurs later in the response is thought to result from increased vascularity of the node. In morphological studies, this second phase of

increased flow is associated with elongation and increased branching of the network of HEV; autoradiography (Fig. 22) following infusion of ^3H -thymidine revealed that the endothelial cells of HEV located nearest to primary follicles became labeled between 3 and 6 days

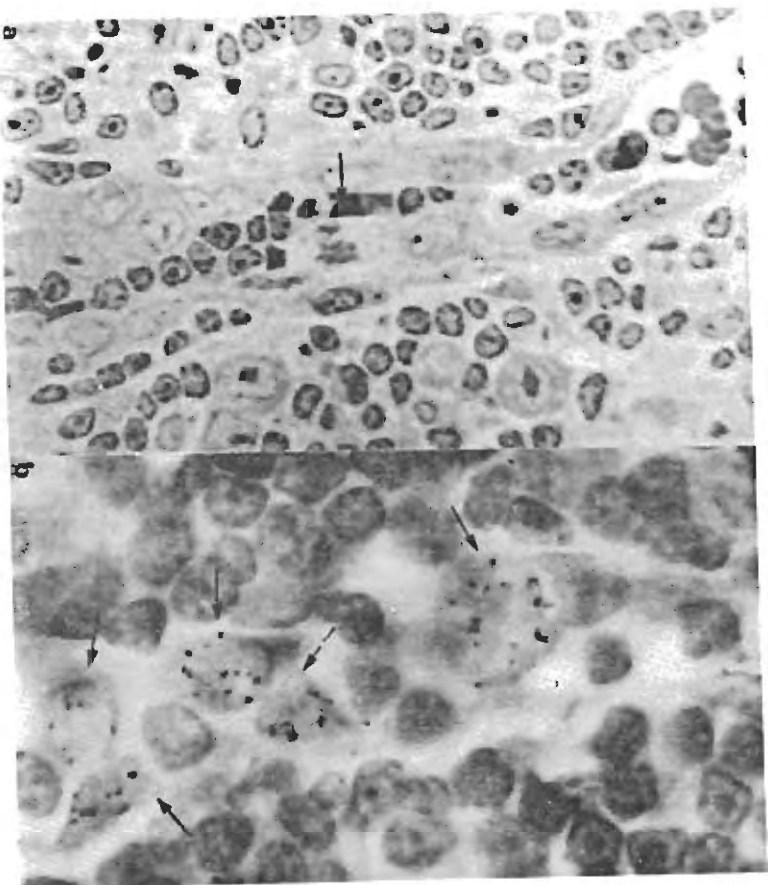


FIG. 22 Proliferation of high endothelial cells (arrows) in proximal segments of HEV 2 and 6 days after alloantigenic stimulation (Anderson *et al.*, 1975b). This endothelial proliferation was identified by appearance of mitotic figures (arrows) (a) and ^3H -thymidine incorporation (arrows) (b).

after alloantigenic stimulation (Anderson *et al.*, 1975). It is likely that these alterations in the microcirculation directly affect transvascular lymphocytic migration into antigen-stimulated lymph nodes initially by increasing the rate of migration across existing HEV and subsequently by enlarging the area of endothelium available for migration.

Several investigators proposed that HEV are specialized vascular units which regulate fluid and cellular exchange between lymphatic tissues and blood. Schulze (1925) described HEV as leaky vessels lined by an endothelium containing stomata for the passage of lymphocytes; Dabelow (1939) later demonstrated that HEV were not highly permeable to particulate dyes. He suggested that the plump endothelium of HEV permitted lymphocyte migration without extravasation because the soft endothelial cells would close around the contours of migrating lymphocytes, thereby permitting them to cross like "ships in canal locks" (Dabelow, 1939). This hypothesis was supported by numerous ultrastructural studies indicating intimate moulding of the highly plastic endothelial cells about lymphocytes migrating through intercellular pathways (Schoell, 1972; Wenk *et al.*, 1974). Recent studies (Anderson *et al.*, 1976; Anderson and Anderson, 1976) suggested that the entire wall of HEV and their surrounding reticular cell sheaths should be regarded as a structural adaptation for controlling fluid and cellular transport. HEV endothelial cells are linked together by discontinuous macular junctional complexes. Lymphocytes may therefore migrate from blood to lymphatic tissue through potential spaces between adjacent endothelial cells. Since these junctional complexes are not broken by the transmigrating cells, the endothelial cell cytoplasm is displaced and moulded about them. As migrating lymphocytes crossed the basal lamina and entered the reticular cell sheath the HEV endothelial cells appeared to secrete new basal lamina to seal the gap. The overlapping plates of the reticular cell sheath were individually linked to structural members of the lymph node reticulum through anchoring filaments and cytoplasmic specializations analogous to those described in lymphatic capillaries by Leak and Burke (1968). This structure could provide additional valve-like functions which would be responsive to changes in intranodal tissue pressures. In nodes subjected to inflammatory stimuli, increased interstitial fluid pressure might cause these plates to separate and release a pulse of tissue fluid and macromolecules which would diffuse between the endothelial cells into the lumen. Such a mechanism permits the formation of an interendothelial "chemotactic" gradient which could induce lymphocytes to begin migrating into the node (Kelly *et al.*, 1972). Ultrastructural studies of the permeability of HEV suggest that this might occur (Anderson and Anderson, 1976), but other studies using slightly different methodologies concluded that HEV were generally leaky vessels (van Deurs *et al.*, 1976). Our study was conducted using Wistar/Furth rats, which are resistant to the altered permeability induced by horseradish peroxidase (HRP), which

was the tracer for both these studies (Cottran *et al.*, 1968), while van Deurs' study was conducted in BALB/c mice which may differ in resistance to the mast cell degranulating effects of HRP.

The endothelial cells of HEV are unlike those of normal venules anywhere in the body (Fig. 23). These cells characteristically exhibit diffuse nonspecific esterase activity in their abundant cytoplasm, almost reaching the staining intensity of monocytes. The presence of a prominent Golgi apparatus, multivesicular bodies, and phagolysosomes in the cytoplasm of HEV cells (which also appear to lack typical Weibel-Palade bodies) suggest that this endothelium may be composed of monocyte-like cells (Anderson *et al.*, 1976). Indeed, the analogy of HEV cells to monocytes can be carried further because the binding of lymphocytes to HEV endothelial cells is morphologically similar to that of cultivated mononuclear cells (Lipsky and Rosenthal, 1973). However, the observation that HEV cells form a continuous lining linked together by junctional complexes which lie upon a basal lamina secreted by these cells is more consistent with a uniquely specialized endothelium than a lining formed by modified monocytes.

d. Lymphocyte emigration into lymph nodes

High endothelial venules were shown by Gowans and Knight (1964) to be the site of large-scale blood-to-lymph emigration of recirculating lymphocytes. Other investigators proposed direct entry of nodal lymphocytes into the blood at these sites, because HEV lumens frequently contain high concentrations of lymphocytes. Subsequent studies indicated that cellular accumulation in HEV lumens was due to selective adhesion of lymphocytes to endothelial surfaces (Figs 7, 8, 24) presumably via receptor molecules located on the tips of microvilli (Anderson and Anderson, 1976). A specific "homing" receptor has not been isolated and characterized, but various studies (Goldschneider and McGregor, 1967) suggest that it may be a sialoglycoprotein, which requires divalent cations and transmembrane connections to a functional cytoskeleton (Anderson *et al.*, 1979) in order to mediate recognition and adhesion of lymphocytes to endothelial surfaces (Ford *et al.*, 1978). Gowans (1959) found that cell viability was essential for "homing", since heat-killed lymphocytes sequestered in the liver and spleen. Preincubation of lymphocytes in trypsin, crude glycosidases and neuraminidase altered the tissue distribution of these cells when infused into normal rates. These experiments were based largely on whole organ counting, and did not establish whether this enzymatic treatment destroyed specific receptor sites or simply made lymphocyte surfaces nonspecifically "sticky". Despite these limitations, Gesner's

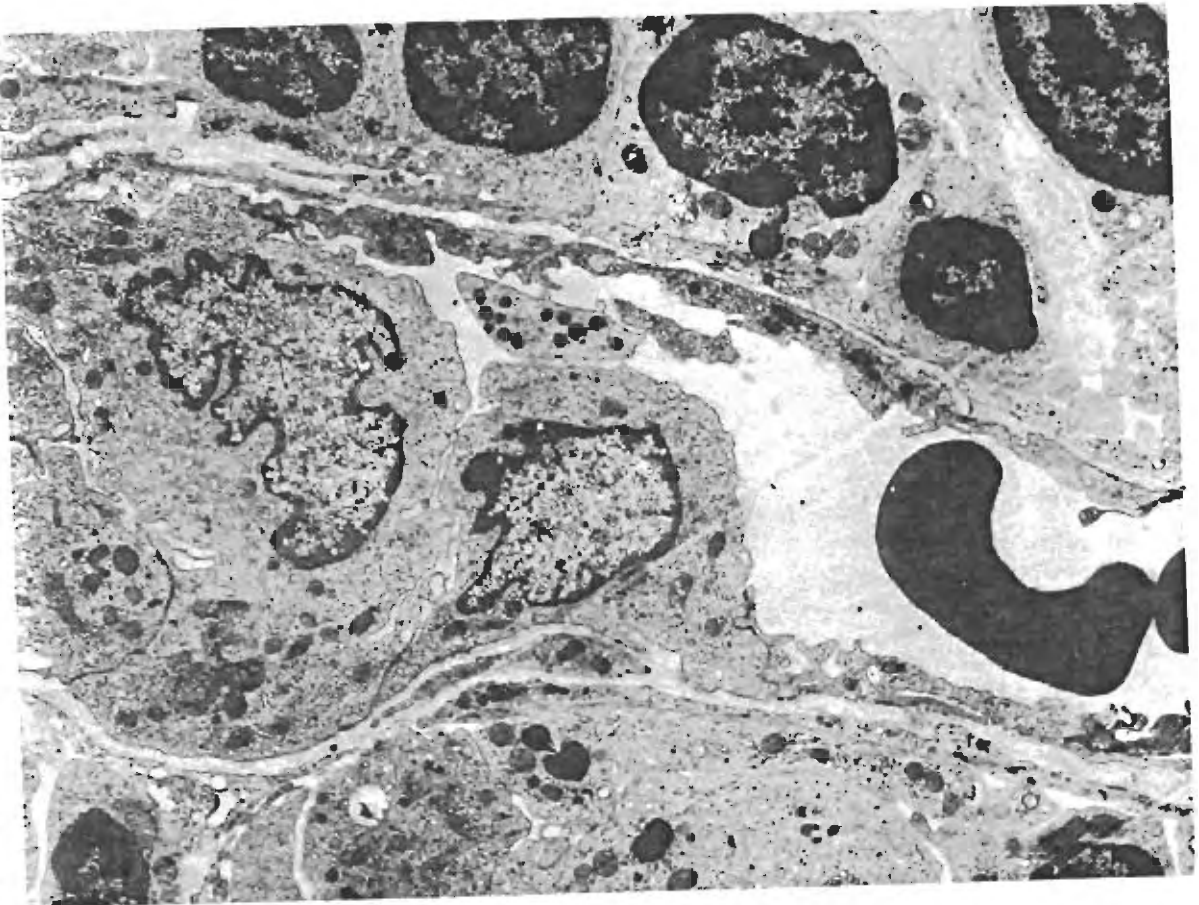


FIG. 23 This electron micrograph shows the abrupt transition from low to high endothelium in proximal segments of HEV.

studies have been widely cited as evidence for specific membrane receptors on lymphocyte surfaces which "recognize" membrane constituents of high endothelial cells to promote selective emigration at this site. *In vitro* models of lymphocyte homing have recently been developed which may help in the chemical and biological characterization of the receptor (Stamper and Woodruff, 1976; de Bono, 1976).

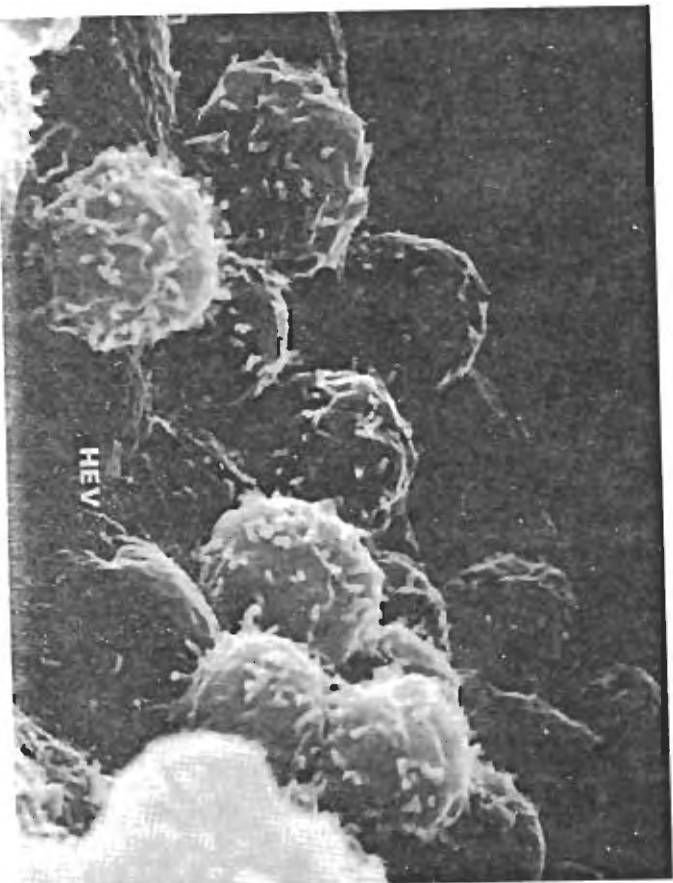


FIG. 24 Circulating lymphocytes adhere to luminal surfaces of HEV prior to emigrating into the lymph node parenchyma.

In addition, studies of lymphocyte binding to frozen sections of lymph node HEV (Butcher *et al.*, 1979) have indicated that the phylogenetic nearness of the source of adhering cells to the species of the substratum (HEV), determines the affinity of binding. That is, human lymphocytes bind better to mouse HEV *in vitro* than do chicken or lizard lymphoid cells.

Transvascular migration in normal lymph nodes is not entirely restricted to lymphocytes, since monocytes, basophils and cells

resembling novel dendritic cells are infrequently found among the lymphoid cells crossing HEV walls. Lymphocytes and monocytes attach to luminal surfaces of HEV, squeeze between the endothelial cells, and enter the perivascular sheath composed of multiple lamellations of reticular cell processes (Fig. 25). After gaining access to the paracortical interstitium, these cells may either crawl along reticular

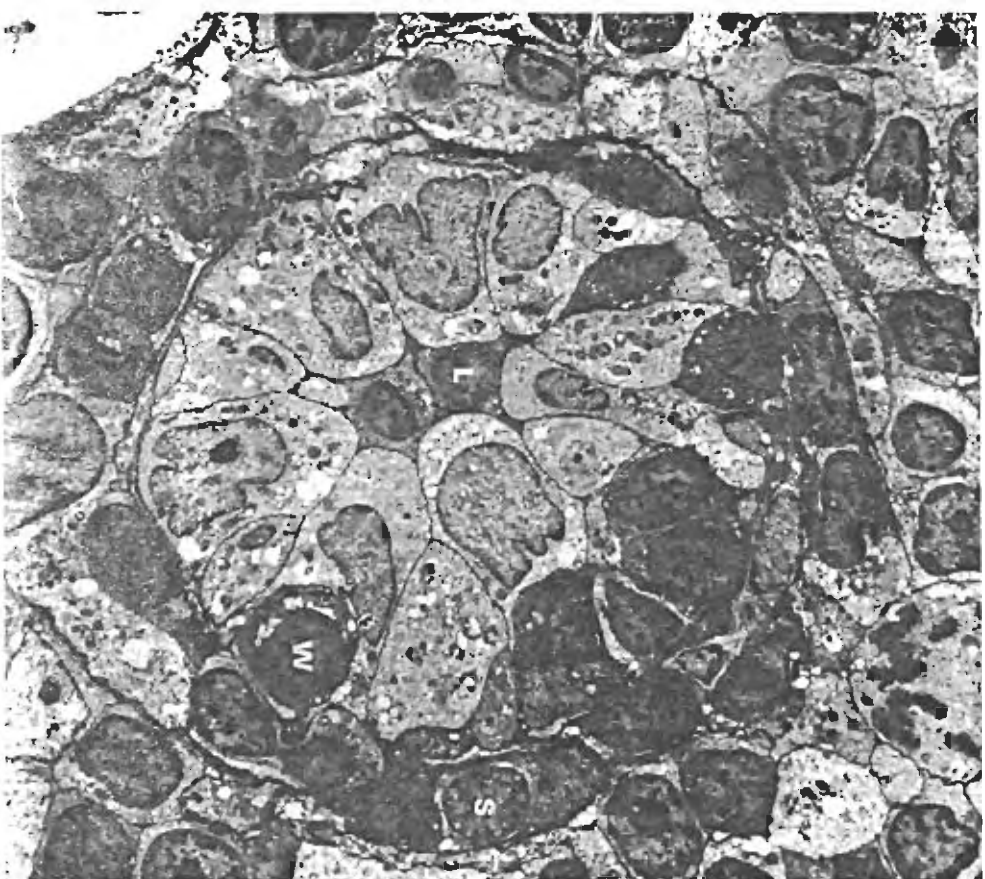


FIG. 25 Migrating lymphocytes can be seen in the lumen (L), wall (W) and sheath (S) of HEV.

fibers toward reactive foci in T or B cell areas or they may cross lymphatic endothelium and enter intermediate sinuses which ultimately delivers them into the efferent lymph. Sprent (1973) and Ford (1975) showed that the mean transit time for migration of normal lymphocytes across lymph nodes was 18 to 20 h for T cells and 28 to 30 h for B cells. The observation that B cells transit lymph nodes more slowly than T cells probably is related to the motile behavior of the respective cells. B cells locomote less frequently and more slowly than T cells. T cells appear to be attracted by short-range factors secreted by B cells, since T cells turn and migrate toward B cells whenever they pass within a 30 to 40 μm radius (Anderson and Warren, 1980). This may obviate the need for equal locomotory activity by B cells in estralisting T-B interactions within lymphatic tissues, since T cells can overtake B cells during their journey from blood to lymph.

Controversy about whether lymphocytes passed through or between HEV endothelial cells (Marchesi and Gowans, 1964) has been resolved to some extent by numerous recent studies using extracellular tracers, serial sections and SEM which showed that lymphocytes enter and cross HEV by migrating between the endothelial cells (Schoeffl, 1972; Wenk *et al.*, 1974; Van Ewijk *et al.*, 1976; Anderson and Anderson, 1976). The controversy had some basis, since lymphocytes deeply indent the endothelial cells as they cross HEV without breaking the macular interendothelial junctions. In acute inflammation, these endothelial junctions separate so that all varieties of blood leukocytes may pass through.

Special permeability characteristics of the HEV wall permit limited intravasation of macromolecules from within the lymph node parenchyma without allowing significant extravasation, even in the presence of transmigrating lymphocytes. This unidirectional permeability derives from the "flap-guarded valve" structure of the HEV wall composed of overlapping endothelial and reticular cells. Diffusion of materials from the lymphatic tissue into the interendothelial spaces may provide a mechanism by which chemotactic gradients are generated. This is especially true under circumstances of antigen stimulation where there is altered blood flow and increased tissue fluid pressures.

2. The spleen

The spleen acts as a large discriminatory filter set across the blood stream. The spleen is largely a meshwork of reticular cells and their fibers containing entrapped macrophages which can remove and

destroy foreign pathogens in the blood and also provide for the selective monitoring and removal of aged and damaged erythrocytes, leukocytes and platelets passing through its interstices.

In immune reactions, the spleen provides for the same processes of antigen trapping, cellular collaboration, lymphocyte proliferation and antibody production seen in peripheral lymph nodes. As a rule, the immune responses to antigens and pathogens invading the blood stream are concentrated within the spleen. In addition, some of the antigen administered locally at distant tissue sites can pass through the regional node and be sequestered within the spleen. The immature lymphocytes mobilized into efferent lymph during immune responses in peripheral lymph nodes pass through the blood and frequently establish secondary residence in splenic cords and sinuses, where the B cells mature into antibody-secreting plasma cells.

a. Structure

The spleen is composed of a dense fibrous capsule with trabeculae extending inward which subdivides the organ into lobules and merges with the reticular meshwork supporting the splenic pulp. The spleen has no cortex and medulla, but its interior is grossly divisible into white pulp, which forms cylindrical collections of lymphocytes about the arteries, and red pulp, which contains erythrocyte-rich blood. The red pulp contains the splenic cords which are continuous partitions of tissue criss-crossed by reticulum lying between the splenic sinuses. The cords contain erythrocytes, lymphocytes, macrophages, granulocytes and numerous antibody-secreting plasma cells. The splenic sinuses are vascular channels between the cords which are lined by endothelial (stave) cells and hoop-like arrangements of basement membrane. The erythrocytes liberated into the splenic cords must squeeze through slits between these endothelial cells to enter the sinuses. The spleen monitors for abnormal erythrocytes by this mechanical sieving and cells which have lost their plasticity are destroyed as they pass from pulp to sinus (Chen and Weiss, 1973).

The spleen is supplied by the splenic artery which passes through the hilus and branches to pass through trabeculae into medium-sized arteries surrounded by a sheath of lymphatic tissue (periarteriolar lymphatic sheath). These vessels terminate in small arterioles which empty into the pulp cords, pulp sinuses or the white pulp and its surrounding marginal zone, which is rich in macrophages. Blood leaves the spleen through a sinusoidal system which connects by gradual transition to pulp veins, trabecular veins and finally the splenic veins. The spleen has no afferent lymphatic vessels, but

does possess efferent lymphatics which arise within the white pulp, pass through trabeculae and exit at the hilus.

b. Lymphocyte emigration into the spleen

Lymphocyte traffic through the splenic red and white pulp is not regulated by the same surface recognition mechanisms which determine selective lymphocyte homing into lymph nodes. Many of the lymphocytes entering the spleen in the arterial blood bypass the white pulp and flow along with other blood elements into the reticulum of the red pulp cords and sinuses to exit via the splenic vein. These cells would be returned to the blood within 2 to 3 h after entering the spleen. However, some small arterioles terminate in sinuses adjacent to the white pulp and others empty directly into the marginal zone. Lymphocytes arriving at these sites move across an antigen-binding interface provided macrophages in the marginal zone (Fig. 26) and enter the PALS. Cells entering here may remain within the spleen for up to 12 h. There is no clear-cut segregation of T and B cells into discrete zones within the splenic white pulp, but follicles and their mantles contain B cells; recirculating T and B cells are found in the PALS. After variable periods of residence here, some recirculating cells exit through the efferent lymphatics, while others appear to move back out via bridging zones into the red pulp sinuses (Mitchell, 1973). The magnitude of lymphocyte recirculation via splenic efferent lymph or venous return remains to be determined. However, if these immunocompetent cells encounter an appropriate antigenic stimulus, they proliferate in the same manner as that seen in peripheral nodes; the B cells can form large germinal centers in this process. A few antibody-secreting cells can be found within the periarteriolar lymphatic sheath, but most of the mature plasma cells appear within the splenic cords in the red pulp following B cell migration through the blood or their direct movement out from the white pulp.

3. The mucosal immune system

The mucosal immune system consists of nonencapsulated lymphoid nodules and diffuse lymphocytic infiltrates which are closely associated with the epithelium lining the intestinal and respiratory tracts. These

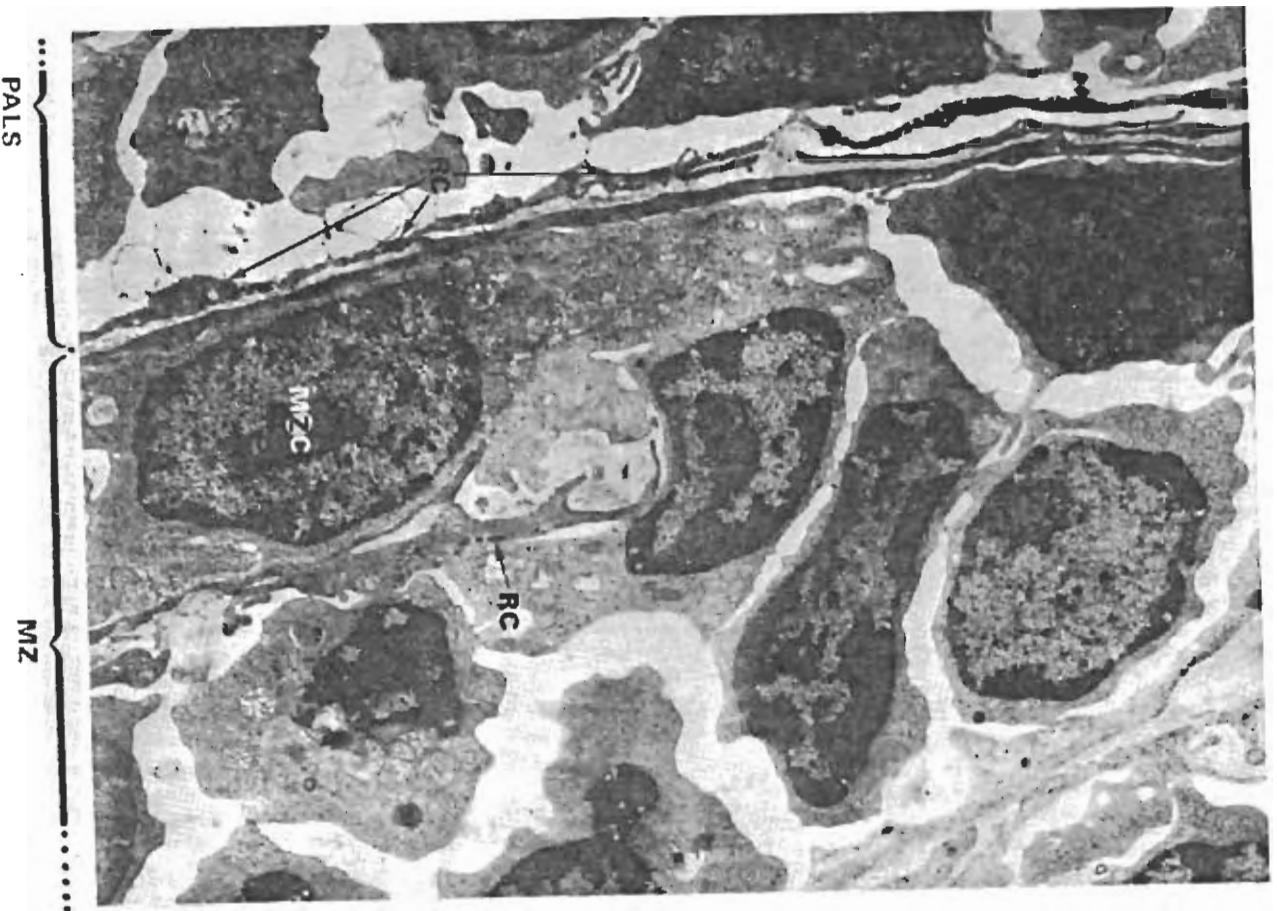


FIG. 26 The marginal zone of the spleen is composed of concentric "onion skin" laminations of reticular cell (RC) processes. Esterase-positive macrophage-like marginal zone cells (MZC) display antigens to lymphocytes which traverse the marginal zone en route to the PALS.

structures provide for the local synthesis and selective transport of secretory IgA antibodies into external secretions promoting local immunity to pathogens and foreign proteins which invade the body through mucosal surfaces. This modified immunoglobulin consists of two IgA molecules linked together by a J chain (MW 15 000) and contains a molecule of secretory component coiled about the Fc portions of the joined immunoglobulin molecules. This structure stabilizes secretory IgA against proteolysis and facilitates its transport across the mucosal epithelium.

Secretory IgA plays an important role in host resistance against many viral diseases by impairing colonization of the mucous membranes and preventing reinfection by the same virus. The efficacy of this process has been well established by the use of oral immunization with live, attenuated viruses, such as the Sabin poliovaccine, which prevents both reinfection and the establishment of the carrier state. Absence of adequate mucosal immunity can result in severe local hypersensitivity reactions when the pulmonary or intestinal epithelium is reinfected by the same viral agent, despite evidence of potent systemic immunity (i.e. the hypersensitivity pneumonitis seen in children immunized parenterally with killed measles vaccine and subsequently infected with live virus). Although secretory IgA probably does not promote direct lysis or phagocytosis of bacteria, it does interfere with bacterial adherence to mucous membranes. When this effect is combined with the normal cleansing actions provided by mucous secretion, cilia beating, cellular desquamation, intestinal motility, etc., secretory IgA can contribute in preventing bacterial colonization within the lung and gut. In addition, there is evidence that secretory IgA can combine with toxins and macromolecules within the gut lumen inhibiting their binding and absorption by intestinal epithelium.

a. Structure

The organized lymphatic tissue components of the mucosal immune system consist of nodular collections of lymphocytes within a reticular meshwork, which are located in the lamina propria and submucosa of the small intestine, appendix, colon, bronchi, tear ducts, salivary glands and mammary glands. Their structure differs from that seen in lymph nodes, because they lack a well-developed capsule, afferent lymphatic connections and a discrete medulla containing cords of plasma cells (Parrott, 1976).

The correlation between structure and function of these organs is best established in the Peyer's patches (Fig. 27). These patches are

discrete, subepithelial collections of lymphoid tissue found along the antimesenteric wall of the intestines. Individual patches are found from the duodenum to the ileum, but are more numerous and larger in

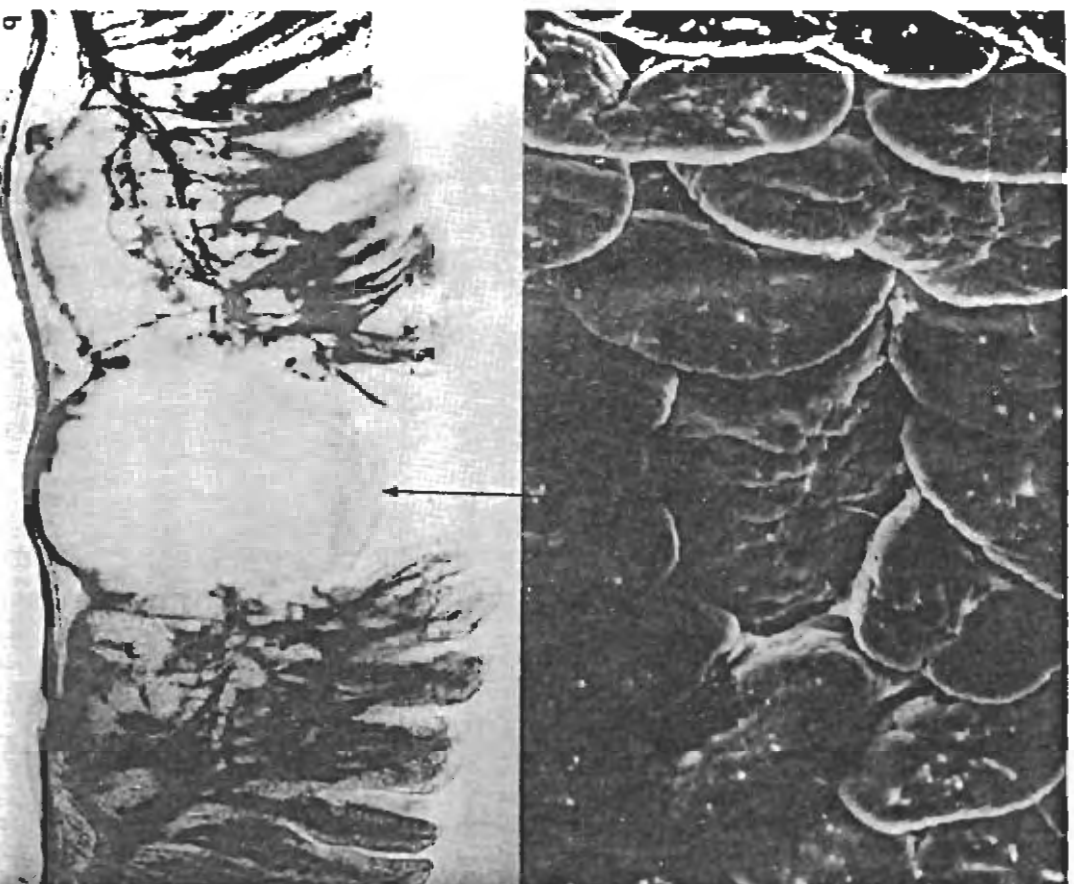


FIG. 27 In Peyer's patches, the epithelium overlying follicles (arrows) is devoid of microvilli but is capable of endocytosis. Lymphocytes often peer out between these microfold cells and are exposed to intact antigens in the gut lumen.

the distal intestinal tract. Each patch contains multiple individual follicles containing dendritic cells, macrophages and proliferating B cells and is surrounded by a mantle of small B cells. The inter-follicular spaces are diffusely infiltrated with small and medium sized lymphocytes, usually of T cell origin. The patches contain specialized HEV and efferent lymphatics which provide for lymphocyte entry and egress. Although Peyer's patches lack true afferent lymphatics, about 30% of the efferent intestinal lymph from the lamina propria lymphatic plexus diffuses through them before emptying into lymphatic trunks which originate beneath the follicles and drain into mesenteric lymphatics. Regardless of how many follicles a patch contains, each is only one follicle thick, providing an intimate association between the follicle and the overlying epithelium. The specialized intestinal epithelium covering each follicle is heavily infiltrated by lymphocytes. These epithelial cells called microfold or M cells are stretched by their burden of interepithelial lymphocytes and lack prominent microvilli; their cytoplasm contains numerous transport vesicles which appear to be specifically adapted to sample intestinal contents (Owen, 1977). These cells endocytose and transport small quantities of antigens across their cytoplasm in vesicles and deposit them among the lymphoid cells which lie above germinal follicles. The direct transport of intact antigens into the patch probably explains how these structures provide immune surveillance in the absence of afferent lymphatics (Bockman and Cooper, 1973). Recent studies have demonstrated remarkably similar transcellular transport of antigen and structural organization in the bronchial associated lymphatic tissues, so these features may be common to all components of the mucosal immune system (Rácz *et al.*, 1977).

Peyer's patches are heavily populated with B lymphocytes, but virtually no IgA secretion occurs in them, despite evidence that many of the B cells in the patch are precommitted for IgA synthesis (Cebra *et al.*, 1977). When antigen is delivered into the patch, these cells, with the help of macrophages and T lymphocytes undergo a clonal burst of proliferation. However, maturation to antibody-secreting cells does not occur in the patch. The immature cells leave through efferent lymphatics, move through the mesenteric nodes and thoracic duct back into the bloodstream. After completing various differentiation steps in the mesenteric node or the spleen, these cells preferentially establish residence at submucosal sites, such as the lamina propria of the intestine (Cebra *et al.*, 1977; Husband and Gowans, 1978). Although still unproven, this distinctive lymphocyte traffic pattern has been variously attributed to the presence of environmental antigens

within intestines or the appearance of IgA dimers on the B cell surface, causing them to localize beneath epithelial cells synthesizing and releasing the secretory component of IgA. Once they have reached these submucosal sites, the B cells mature into plasma cells.

b. Migration cycles of lymphocytes associated with mucosal immunity

³H-Uridine-labeled TDL emigrate into peripheral lymph nodes and accumulate to a peak over an 18-22-h period (Fig. 28). These same lymphocytes enter the thymic-derived area of Peyer's patches via HEV

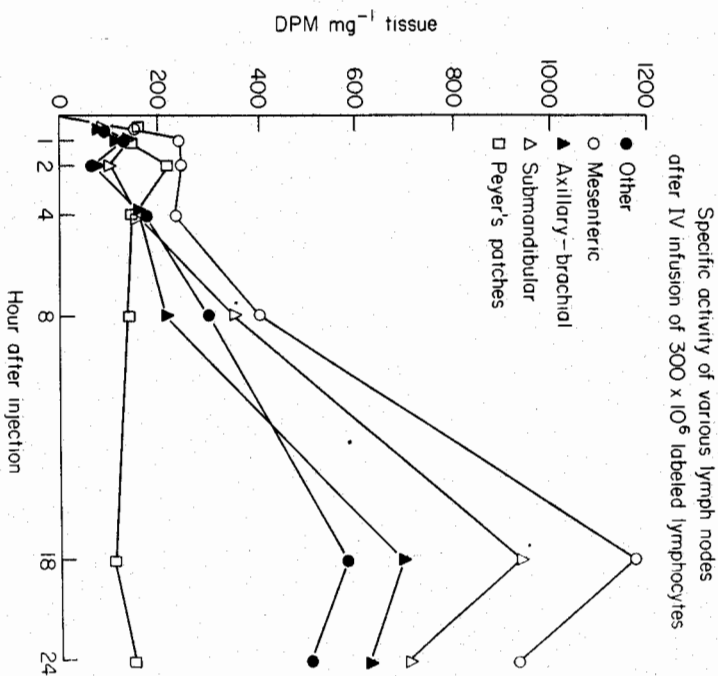


FIG. 28 The accumulation of trafficking lymphocytes in lymph nodes increases gradually over an 18-hour period. The rate of this accumulation is presumably proportional to the degree of immunological activity of the node. However, traffic to the Peyer's patch does not seem to be regulated by the same mechanisms as it is in lymph nodes. Labeled lymphocytes entering Peyer's patches peak by 2 hours and then they must leave, presumably to the mesenteric lymph node via lymphatics.

and rapidly exit via efferent lymphatics which cross the muscularis propria at the bases of germinal follicles. These cells appear in the subcapsular sinuses of mesenteric lymph nodes within 1-1/2 h and significantly contribute to the rate of accumulation of labeled lymphocytes in these nodes. Since mesenteric lymph nodes also receive lymphocytes via paracortical HEV, the steady state of lymphocyte entry into these nodes is normally two to three times that of peripheral lymph nodes. At these same time periods none of the primary or secondary chains of peripheral lymph nodes contain radiolabeled cells in their lymph sinusoidal systems. This observation is consistent with the segregation of antigen-triggering and subsequent maturation of IgA precursors which are said to occur in the Peyer's patches and mesenteric lymph node, respectively. Since long-lived recirculating T cells do not remain in the Peyer's patches long enough to participate in the maturation steps of IgM-bearing IgA precursor cells, it is possible that the T cells, exposed to identical antigens as the B cells by passage through the Peyer's patches, catch up with the IgA precursors in the lymph sinuses of the mesenteric node. Passage of these appropriately "helped" B lymphoblasts through the lymph to the blood and ultimately the gut completes the cycle of migration, since the "secondary" B cells lose their motility and lodge as antibody-secreting cells in the lamina propria.

The frequency and potential of IgA "memory" cells in TDL has not yet been well studied. However, Husband and Gowans (1978) found a subpopulation of presumably recirculating cells in TDL of rats 3 to 4 days after secondary intraluminal challenge which, when transferred to rats bearing an isolated intestinal loop containing antigen, localized in the loop, divided there and expressed IgA antibody. Thus, one aspect of the secretory antibody response may be the release of long-lived IgA precursors by Peyer's patches, which circulate through and/or lodge in mucosal tissue. Local reapplication or persistence of the antigen may then initiate their division and maturation to IgA cells at the site, presenting the aspect of a "true local secondary response".

Either the mucosal follicles can selectively accumulate IgA precursors, which differentiate elsewhere, or these cells are generated in the special milieu of the mucosal follicles. Cebra *et al.* (1977) proposed that the special feature of mucosal follicles that promotes the generation of IgA-only clonal precursors in an environment that allows antigen-driven division without maturation to plasma cells. The feature of the Peyer's patches environment that might promote this situation could include inaccessibility of T "helper" cells, excessive presence of T "suppressor" cells or humoral factors (Faulk *et al.*, 1971;

Waksman *et al.*, 1973; Kamini *et al.*, 1974; Ngan and Kind, 1978). This process might operate on the B cell which generates clones expressing both IgM and IgA.

Virtually all the IgA found in external body secretions is produced by local synthesis within plasma cells. After the IgA molecules are synthesized and assembled, the J piece is added during secretion and release of the dimers into submucosal tissue. These dimers diffuse across the basement membrane where they are coupled to secretory components which are synthesized and bound to the surface of epithelial cells. The resulting complex is endocytosed and transported through the epithelial cell cytoplasm to be secreted into the intestinal or bronchial lumens. In the lumens, it can bind with the appropriate antigen or be lost from the body during normal passage of the intestinal contents and mucus to the exterior. In addition, some IgA may be reabsorbed into the blood, removed by the liver and secreted back into the intestine with the bile, providing a form of enterohepatic circulation which may greatly augment intestinal immunity (Orlans *et al.*, 1978).

4. Peripheral sites of chronic inflammation

Antigens presented in a form not easily removed by cells of the mononuclear phagocyte system, e.g. emulsified in a non-degradable oily base, result in the formation of chronic inflammatory foci. These lesions possess follicular and diffuse lymphocytic infiltrates in addition to collections of foam, epithelioid and giant cells and other monocyte components of granulomata (Spector and Willoughby, 1963). Similar collections of diffuse lymphoid tissue may be seen: in lesions resulting from infections by facultative intracellular organisms of viral, bacterial and parasitic origin; in tissues exposed chronically to microbes present in the fluids which bathe them, such as the bladder or renal pelvis of patients with chronic bacilluria due to pyelonephritis; in organs which are undergoing chronic rejection following transplantation; and in organs attacked by autoallergic phenomena, such as Hashimoto's thyroiditis, allergic orchitis and allergic encephalomyelitis.

All of the above chronic inflammatory lesions contain structural elements which are similar to those of non-encapsulated lymphatic tissue. The inflammatory infiltrate which succeeds neutrophils in these sites is composed predominantly of monocytes, macrophages and lymphoid cells. While monocytes and lymphocytes usually arrive at the site simultaneously, large-scale lymphoid cell emigration occurs only after additional microenvironmental structures are generated. Chronic

inflammation typically develops in connective tissue sites which are well supplied by lymphatic channels. The inductive stimulus, which is possibly provided by a number of factors released by monocytes and lymphoblastic cells, results in proliferation of fibroblasts and lymphatic and blood vascular endothelium (Fig. 29). Specialized vascular segments develop by mitotic division, which morphologically, histochemically and functionally resemble HEV of lymph nodes (Astrom *et al.*, 1968; Smith *et al.*, 1970; Graham and Shannon, 1972). Such new vessels lined by plump, esterase-positive endothelial cells arise in chronic inflammatory foci prior to the appearance of dense aggregates of small lymphocytes. Circulating lymphocytes home to these vessels, emigrate from the blood and accumulate temporarily in the lesion before passing into afferent lymphatics and regional lymph nodes. Lymphoblastic cells also enter the lesion at such sites and differentiate into plasma cells or participate in the formation of germinal follicles. The antibody specificities of these cells are frequently unrelated to the antigens present at the site.

This large-scale lymphoid cell traffic through sites of chronic inflammation has been documented in studies where the outputs of cannulated afferent lymphatics draining adjuvant-induced granulomata were measured (Smith *et al.*, 1970) or where the distributions of transfused ^3H -uridine-labeled lymphocytes were traced in tissues by autoradiography (Anderson and Reynolds, 1979). In the former studies, the afferent lymph output in sheep inoculated with Freund's adjuvant reached 3.1×10^7 lymphocytes per hour, which is nearly equal to the output of a normal lymph node, and is at least ten times that of normal afferent lymph. Increased lymphocyte traffic via the granuloma site persisted through the 70 days of the study and probably would have continued as long as the lesion was present. In the latter study the lymphocytes present in the afferent lymph were identified as predominantly B cells. This augmentation of B cell traffic to the regional lymph node via the granuloma site correlated with the pronounced increase in antibody production and with the altered ratios of T and B lymphocytes found in these nodes. The enriched B cell populations present in the afferent lymph may have been recruited from the circulation, but their formation in germinal follicles is also possible.

Macrophages in the lesion, which are unable to completely remove the antigen-containing material, become activated and secrete mediators which affect the local microenvironment and that of the regional lymph nodes by stimulating lymphocyte division, chemotaxis of monocytes and lymphocytes, vascular permeability, blood flow and

cell proliferation (Umanne *et al.*, 1976). These peripheral sites of chronic inflammation resolve when all the antigen is neutralized and eliminated by phagocytes; they may, therefore, serve a beneficial

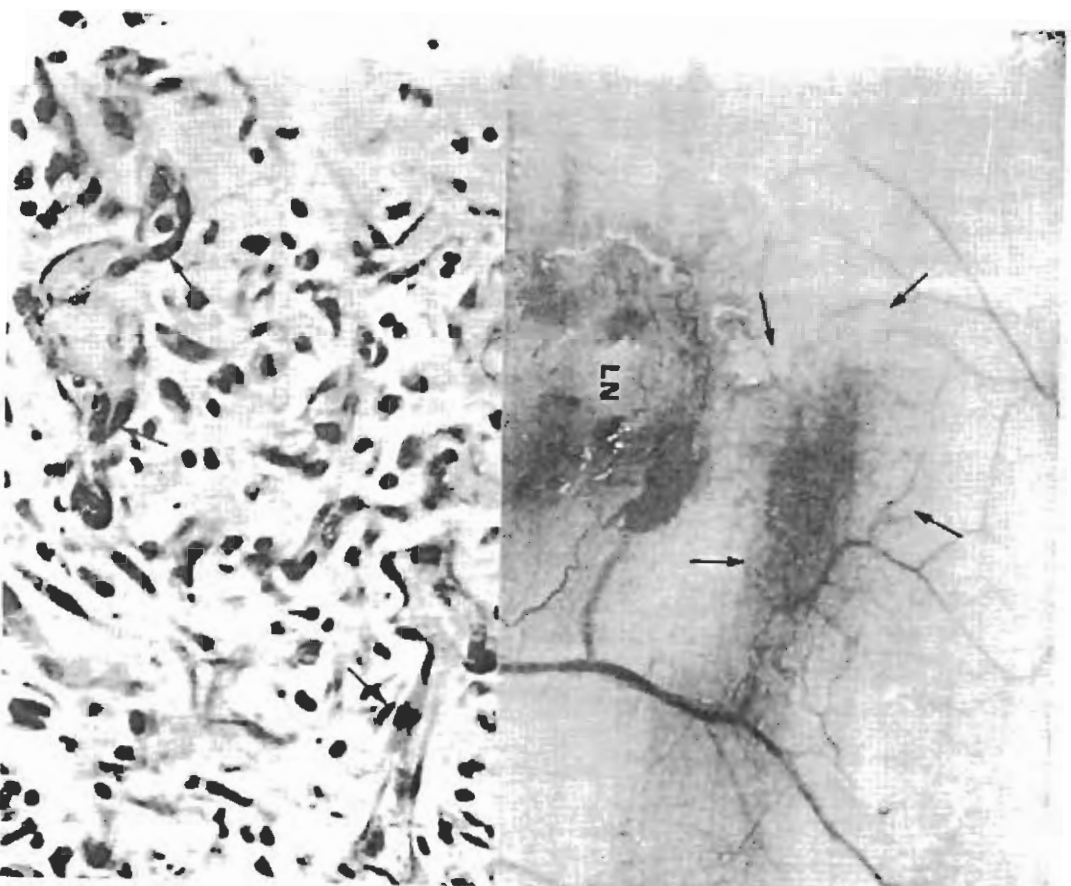


FIG. 29 Chronic inflammation is associated with hyperemia and vascular proliferation. This is illustrated here by undersurface of rat skin, which shows these vascular changes overlying the site of injection (arrows) of a water soluble adjuvant. The regional lymph (LN) is located nearby and also has hyperemic vessels along the afferent lymphatics.

purpose by establishing new reticular microenvironments which serve the same antigen- and cell-sorting functions as peripheral lymphatic tissues. However, these foci also cause discomfort and disease when the antigen is (a) a normal constituent of the host, (b) not degradable or neutralizable or (c) capable of changing its antigenic determinants in response to immunological pressure. In addition, hydrolytic enzymes which may be released in the course of phagocytosis may damage other neighboring structures, such as the articular surfaces of joints in rheumatoid arthritis and the arthritis associated with autoallergic diseases.

VI. Circulation of lymphocytes during an immune response

Successful initiation of a specific immune response requires that the lymphocytes must engage and bind the appropriate antigen with its surface receptor. Studies in non-immune animals have shown that only a tiny minority of the lymphocytes present within a single lymph node are capable of reacting with a given antigenic determinant. If these cells were static or their movements were randomly sorted throughout the body, the likelihood of chance collisions between reactive lymphocytes and the appropriate antigen displayed on an accessory cell would be very remote. However, this is clearly not the case *in vivo*, where immunocompetent T and B cells continually recirculate between blood and lymphatic tissues (Sprent, 1973). Long-lived lymphocytes flowing in the blood show a unique "homing instinct" for lymph node HEV. These cells cross HEV and emigrate into the reticular meshwork, where they crawl along reticular cell surfaces, collide with macrophages and other lymphocytes and either stay to initiate proliferation or leave.

Lymphocytes recirculate through other lymph nodes and mucosal lymphatic tissues via HEV, providing a constant form of surveillance by immunocompetent lymphocytes that move through antigen-binding meshworks. This phenomenon probably enables a small depot of antigen to recruit a large number of antigen-specific cells from the body's lymphocyte pool.

When an individual lymphocyte encounters an appropriate antigenic stimulus within the node, it binds the antigen, interacts with other cell types and is trapped in the node, where it gives rise to a clonal burst of proliferation and differentiation of lymphocytes which mediate immune responses. Many of the immature lymphocyte progeny leave the stimulated node 48-100 h after antigen exposure and disseminate to distant nodes, the spleen and other tissues, where they

can mature into specific effector cells. The B cells activated in this response move into the medullary cords of regional and distant nodes, where they mature into antibody-secreting plasma cells (Frost *et al.*, 1976).

Although T and B lymphocytes emigrate from the same segments of HEV, they are sorted by unknown mechanisms within the nodal parenchyma. The T lymphocytes establish residence in the deep cortex for relatively short time intervals before moving out into the efferent sinuses. B cells emigrate into the superficial cortex and probably remain there for longer periods before exiting via the sinusoidal pathways. This migration pattern appears to permit the T and B cells to interact with antigen-binding macrophages and engage in cellular collaboration before they redistribute into their respective zones within the nodal cortex.

Other factors also influence lymphocyte traffic patterns in the body. The ability of T cell subpopulations to recirculate appears to depend upon their state of maturation (i.e. at least some effector T cells leave the circulation at random to enter sites of inflammation). B lymphocytes display similar variations in their emigration patterns. The immature B cells appearing shortly after antigenic challenge may leave the node, but they frequently lodge in the spleen and do not recirculate through the thoracic duct. However, "memory B cells" arising at the late stages of an immune response do recirculate in a typical manner between blood and lymph (Ponzo *et al.*, 1977). The B cell precursors of IgA secretion also possess distinct and quite different migration pathways. This circuit include generation of precursor progeny in Peyer's patch follicles, which complete various maturational steps in the mesenteric node and spleen before finally lodging in the lamina propria of the gut as IgA-secreting plasma cells.

In non-stimulated animals, lymphocyte recirculation is characterized by a balanced flux of cellular traffic across afferent and efferent terminals of lymphatic tissues (Fig. 30). This kinetic equilibrium is rapidly distorted in the regional nodes draining sites of inflammation, infection or antigenic challenge (Hall and Morris, 1965; Cahill *et al.*, 1976). This is accompanied by a rapid increase in lymphocyte accumulation within the lymph node cortex, which is not associated with cellular replication. Such nodal enlargement probably reflects the combined result of increased lymphocyte traffic into the node and decreased egress, or markedly increased entry with normal rates of exiting. These early changes are believed to be produced, in part, by changes in blood flow and by release of secretory factors and lysosomal enzymes from activated macrophages, which alter lymphocyte surface

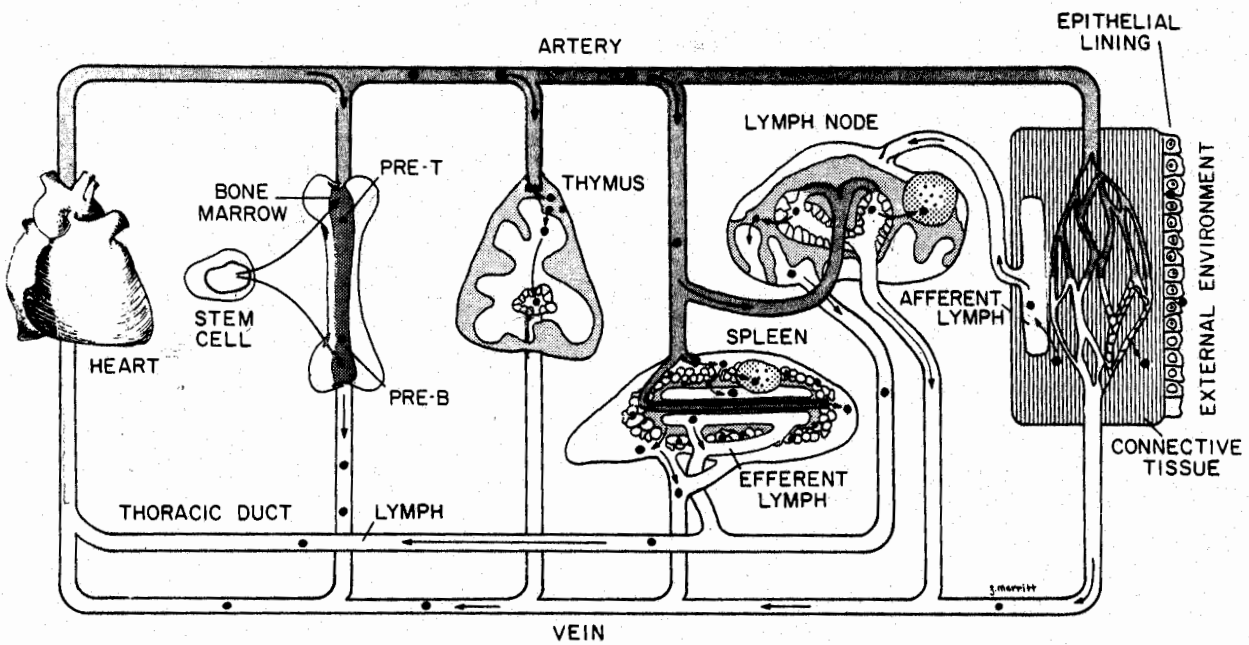


FIG. 30 This diagram depicts the circuits of lymphocyte traffic explained in the text.

adhesiveness and transit times within the node (Unanue *et al.*, 1976). As this early sequestration of recirculating lymphocytes within the stimulated node subsides, blast cell transformation and mitotic activity appear in the T and B cell zones of the cortex, reflecting the antigen-dependent cellular proliferation.

The specificities of the lymphocytes entering lymphatic tissues in the immediate 6- to 48-h period following stimulation are largely unrelated to the antigen which initiated the response. In fact, similarly non-specific lymphocytic accumulations can be induced by adjuvants which themselves are poorly immunogenic. However, as the sequestered lymphocytes are released into the efferent lymph, they are depleted of cells specifically reactive to the priming antigen (Rowley *et al.*, 1977). The retention of antigen-specific lymphocytes in regional lymph nodes has been termed "specific recruitment", and was documented using double-label techniques. Specific memory cells generated *in vivo* in the presence of ^{14}C -thymidine were mixed with equal numbers of ^3H -thymidine-labeled control memory cells and subsequently transfused to immunized syngeneic recipients. The relative accumulation of specific versus non-specific cells was measured in regional and contralateral nodes by scintillation spectroscopy of extracted DNA. These kinds of studies clearly demonstrated increased traffic of specifically reactive cells in antigen-draining nodes which withstood reciprocal specificity controls (Sprent *et al.*, 1971; Thursh and Emeson, 1972; Parrott and Ferguson, 1974). The numbers of labeled immunospecific cells available to lodge selectively were small enough to tax the resolving power of the assays, but studies of efferent lymph indicated that specific cell traffic remained elevated throughout the immune response. Specifically reactive lymphocytes enter the efferent lymph in maximal numbers between 72 and 100 h. Peak accumulations of antigen-reactive lymphocytes correlated chronologically with early formation of reactive centers in the cortex and proliferation of new segments of HEV (Anderson *et al.*, 1975), suggesting that these vascular structures may have some function in selective recruitment, but lymphocyte chemotactic factors or modified antigens secreted by macrophages may also favor the migration of specific cells to HEV.

The increased migration of lymphocytes into antigen-stimulated lymphatic tissues is not restricted to long-lived recirculating lymphocytes. Lymphocytes in the cortex of the thymus undergo enhanced proliferation within the first three days following peripheral antigen inoculation. The systemic stimulus which causes thymocyte proliferation is presently unknown, but these newly formed cells leave

the thymus and accumulate in the marginal zone of the spleen, Peyer's patches and some lymph nodes (Durkin *et al.*, 1978). These cells comprised predominantly of $Ly^{(1+,2+,3+)}$ cells may be precursors of antigen-specific suppressor cells which require T cell help in order to complete their differentiation. Adult thymectomy, which removes the short-lived, cortical thymocytes but not the long-lived recirculating cells, prevents the formation of Freund's adjuvant-induced T-suppressor cell populations, further supporting the suggestion that the cortical thymocytes which lodge in the spleen are precursors of suppressor cells. As early as 6 days after immunization, the numbers of antigen-specific suppressor cells begin to increase until the immune response is terminated.

These changes in lymphocyte traffic combined with local proliferation of immunoreactive cells are responsible for the biphasic 2- to 6-fold enlargement of regional lymph nodes draining sites of antigen inoculation or infection. In the absence of additional free antigen, the suppressive effects of anti-idiotypic antibodies and/or suppressor cells eventually result in gradual reduction in traffic and proliferation. It may take a month or more for the regional lymph node to return to its normal size and physiologic activity. Following secondary exposure to antigen, the kinetics of lymphocyte traffic and immunocyte proliferation in the regional and distant lymph nodes are greatly accelerated, possibly because of the dissemination of foci of immunological memory to nearly all the lymphatic tissues of the body.

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